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Research Paper

Differential expression of pro-inflammatory and anti-inflammatory cytokines during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves

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ABSTRACT

The objective was to compare the mRNA expression of pro-inflammatory (TNF-α, IL-1β, IFN-γ, IL-2, IL-12, IL-15) and anti-inflammatory (IL-4, IL-10, TGF-β) cytokines, after experimental infection with low or high virulence noncytopathic (ncp) bovine viral diarrhea virus (BVDV). Thirty BVDV-naïve, beef calves were intranasally inoculated with low (LV; $n = 10$, SD-1) or high (HV; $n = 10$, 1373) virulence ncp BVDV or with BVDV-free cell culture medium (Control, $n = 10$). Calves were euthanized on day 5 post-inoculation, and tracheobronchial lymph node and spleen samples were collected for mRNA expression through quantitative-RT-PCR. mRNA levels of pro-inflammatory (TNF-α, IL-1β, IL-2, IFN-γ) and anti-inflammatory (IL-4 and IL-10) cytokines were up-regulated in tracheo-bronchial lymph nodes of HV, but not in LV, compared to the control group ($P < 0.05$). IL-12 mRNA level was up-regulated in tracheo-bronchial lymph nodes of both LV and HV groups ($P \leq 0.05$). A significant up-regulation of IL-15 mRNA was observed in tracheo-bronchial lymph nodes for LV calves ($P < 0.002$), but not for HV calves. Experimental inoculation with BVDV-2 1373 stimulated significant mRNA expression of pro-inflammatory and anti-inflammatory cytokines. In contrast, inoculation with BVDV-1a SD-1 only resulted in up-regulation of IL-12 and IL-15 mRNA, which is associated with activation of macrophages and NK cells during innate immune response.

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1. Introduction

Most acute bovine viral diarrhea virus (BVDV) infections are subclinical, transient and self-limiting. However, BVDV can potentiate infections by other bacterial and viral agents resulting in the bovine respiratory disease complex (Baker, 1995), one of the most important infectious diseases in commercial feedlot production. This increased susceptibility to secondary infections is a consequence of the ability of BVDV to cause immunosuppression and synergism with other infectious agents (Kapil et al., 2005). Acute infections with some virulent BVDV-2 strains may cause severe clinical disease characterized by fever, depression, respiratory signs, diarrhea, lymphopenia, thrombocytopenia and high mortality rate (Brock et al., 2007; Liebler-Tenorio et al., 2003). Thus, the wide range of clinical presentations following acute BVDV infections depends on the viral strain virulence and the immune status of the animal (Ridpath et al., 2006). Previous studies have shown that high virulence BVDV strains induced a significantly

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more severe and longer lymphopenia and lymphoid depletion than low virulence BVDV strains (Kelling et al., 2002; Liebler-Tenorio et al., 2002, 2003). These results indicated a possible relationship between the strain virulence and immunosuppression caused by BVDV in susceptible cattle. In addition to reduction in leukocyte counts, BVDV may cause immunosuppression by impairing the leukocyte function (Baigent et al., 2004; Charleston et al., 2001; Glew et al., 2003; Jensen and Schultz, 1991; Lamontagne et al., 1989; Liu et al., 1999; Potgieter, 1995; Schweizer et al., 2006). A Th1/Th2 polarization, previously described in mice and humans (Diebold, 2008; Gaffen and Hajishengallis, 2008; Mosmann and Coffman, 1989; Paul and Zhu, 2010) is not well defined in ruminants (Estes and Brown, 2002). Some studies have demonstrated the development of a Th1 immune response (e.g. IL-2 and IFNγ production) after BVDV infection (Charleston et al., 2002; Lee et al., 2008). Conversely, others have shown the establishment of a Th2 type immune response (e.g. IL-4) after infection with ncp BVDV, which could inhibit the Th1 response and cause immunosuppression leading to secondary pathogen infections (Rhodes et al., 1999). A recent study to determine the memory response of cytokine expression in peripheral blood mononuclear cells from cattle after stimulation with ncp BVDV was not consistent for demonstrating that BVDV induces a Th1 or Th2 biased immune response (Waldvogel et al., 2000). In the present study, it was hypothesized that acute infection with high or low virulence BVDV strains can differentially regulate the expression of pro-inflammatory (Th1) and anti-inflammatory (Th2) cytokines in BVDV-naïve calves, which would reflect differences in their ability to cause immunosuppression and clinical disease. The objective of this study was to compare the mRNA expression of cytokines involved in pro-inflammatory (TNF-α, IL-1β, IFN-γ, IL-2, IL-12, IL-15), and anti-inflammatory (IL-4, IL-10, TGF-β) responses after experimental infection of BVDV-naïve beef calves with low (strain SD-1) or high (strain 1373) virulence ncp BVDV, to determine if there is an association between BVDV virulence and immunosuppression.

2. Methods

2.1. Animals

A total of 30 beef calves (7 months of age) were enrolled in this study. All calves were clinically normal, free of BVDV based on virus isolation from serum and immunohistochemistry of ear notch biopsies and seronegative to both BVDV-1 and BVDV-2 based on serum virus neutralization assays performed at serum dilution of 1:2.

2.2. Experimental design and treatments

Calves were randomly assigned to one of three treatment groups: LV ($n = 10$): animals challenged with a low virulence ncp BVDV-1a SD-1. HV ($n = 10$): animals challenged with a high virulence ncp BVDV-2 1373. Control ($n = 10$): animals inoculated with BVDV-free cell culture medium. All calves were housed together at a BVDV-free farm prior to inoculation. As groups were inoculated, they were separated and transported to the BVDV isolation farm, located at 1.6 miles away from the BVDV-free farm, except for the control group which stayed in the BVDV-free farm throughout the experimental period. Additionally, in order to avoid shedding and exchange of BVDV among groups and undesired BVDV infection in the control group, the study was performed during three different rounds of viral inoculation-necropsy, spatially and temporally separated as previously described (Palomares et al., 2013). At both farms, calves within each group (LV, HV or control) were housed together (same pasture) and allowed to eat and drink from the same feeders and water troughs. Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Auburn University.

2.3. BVDV challenge inoculation

Calves were intranasally inoculated with a low (strain SD-1) or high (strain 1373) virulence ncp BVDV isolates. The inocula used consisted of 5 mL of cell culture supernatants containing 1.3×10^5 $CCID_{50}$/mL of BVDV-1a strain SD-1 and 1.3×10^5 $CCID_{50}$/mL of BVDV-2 strain 1373 of inoculum in Dulbecco's Modified eagle's medium (DMEM).

2.4. Sampling

From each calf, blood samples were collected on days 0, 4, and 5 after BVDV challenge for determination of the leukocyte counts. On day 5 post challenge, animals were euthanized and tissue samples of spleen and tracheo-bronchial lymph nodes were collected and stored at −80 °C as previously described (Palomares et al., 2013).

2.5. Virus isolation

Virus isolation from spleen samples was performed to confirm BVDV infection in the inoculated animals. For virus isolation, 250 μL of each macerated sample was added to individual 25 cm^2 tissue culture flasks containing a monolayer of Madin–Darby bovine kidney (MDBK) cells and tested in 96-well plates using immune-peroxidase staining as previously described (Palomares et al., 2012).

2.6. mRNA gene expression analysis

Spleen and tracheo-bronchial lymph node tissue samples were disrupted and homogenized using a bullet blender machine [1]. Total RNA for quantitative real time RT-PCR (*q*RT-PCR) was extracted and purified from spleen and tracheo-bronchial lymph node samples using an RNA extraction kit [2], according to the manufacturer's protocol. Synthesis of cDNA was performed using a first cDNA strand reverse transcription kit [3] according to the manufacturer' protocol. Then, *q*RT-PCR was performed in 20 μL reactions to amplify the target gene sequences and determine the mRNA level using a real time

[1] Next Advance® Averill Park, NY, USA.
[2] RNeasy mini kit QIAGEN®, Valencia, CA, USA.
[3] Roche®, Indianapolis, IN, USA.