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Lymphocytopathogenic activity in vitro correlates with high virulence in vivo for BVDV type 2 strains: Criteria for a third biotype of BVDV

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Abstract

Two biotypes of bovine viral diarrhea viruses (BVDV), cytopathic (cp) and noncytopathic (ncp), are recognized based on their activity in cultured epithelial cells. Biotype does not correlate to virulence in acute infections as BVDV strains associated with severe acute BVD outbreaks are all noncytopathic based on their growth characteristics in cultured epithelial cells. Previous studies have shown that acute infections with highly virulent BVDV result in depletion of cells in lymphoid tissues. In this study, flow cytometry demonstrated that infection with a highly virulent BVDV strain was associated with a pronounced reduction in circulating white blood cells (WBC) and increased numbers of apoptotic and necrotic circulating WBC in vivo. Infection with low virulence BVDV did not result in a significant increase in death of circulating WBC. Thus, there appeared to be a correlation between depletion of circulating WBC and virulence. To study the interaction of BVDV strains with lymphoid cells in the laboratory, we developed an in vitro model that used a bovine lymphoid cell line (BL-3 cells). Using this model, it was found that while BVDV strains are segregated into two biotypes based on their activity in cultured epithelial cells, they may be segregated into three biotypes based on their activity in cultured lymphoid cells. These three biotypes are noncytopathogenic (no obvious effects on the viability of either cultured epithelial or lymphoid cells), cytopathogenic (cytopathic effect and cell death in both cultured epithelial and lymphoid cells within 48 h of infection) and lymphocytopathogenic (no effect on cultured epithelial cells, however, cell death in cultured lymphoid cells is observed within 5 days of infection). The proposed lymphocytopathic biotype correlates with high virulence in acute infections in vivo. Cell death caused by the lymphocytopathogenic biotype was not associated with changes typically seen with cytopathic viruses grown in cultured epithelial cells (e.g. changes in processing of the NS2/3 protein observed within 24 h post infection, crenation and breakdown of cell integrity within the first 48 h post infection). These data suggest that the cytopathic effect induced in cultured lymphoid cells by a ncp highly virulent BVDV strain may occur by a different mechanism than the cytopathic effect induced by cp BVDV strains.
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Keywords: Bovine viral diarrhea virus; Biotype; Virulence in vivo; Cytopathogenic; Noncytopathogenic; Lymphocytopathogenic

1. Introduction

Bovine viral diarrhea viruses (BVDV) are endemic in ruminant populations worldwide. Like other members of the pestivirus genus, within the Flavivirus family, they are small enveloped viruses with a single stranded RNA genome (Gillespie et al., 1960; Heinz et al., 2000; Lee and Gillespie, 1957; Lindenbach and Rice, 2001). Two genotypes and two biotypes of BVDV have been recognized (Gillespie et al., 1960; Lee and Gillespie, 1957; Pellerin et al., 1994; Ridpath et al., 1994). The two genotypes are called BVDV1 and BVDV2 and are now recognized as distinct species within the pestivirus genus (Heinz et al., 2000). The two biotypes, cytopathogenic and noncytopathogenic, are based on the activity of the BVDV strain in cultured epithelial cells (Gillespie et al., 1960; Lee and Gillespie, 1957). The practical significance of biotype is that, in vivo, noncytopathogenic viruses may establish persistent infections following in utero infection but cytopathogenic viruses do not. Noncytopathogenic viruses predominate in nature. Cytopathogenic viruses are relatively rare and usually found in association with outbreaks of mucosal disease, a relatively infrequent but highly fatal form of BVDV infection (Houe, 1995, 1999, 2003;

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Lindberg, 2003). Cytopathology in vitro does not correlate with virulence in vivo (Bezek et al., 1994) and the most clinically severe form of acute BVDV infection is associated with noncytopathogenic virus (Bolin and Ridpath, 1992; Carman et al., 1998; Corapi et al., 1990, 1989; Pellerin et al., 1994; Ridpath et al., 1994).

The effects of acute infection with BVDV may range from clinically unapparent to clinically severe. Clinical presentation following acute infection is dependent on the viral strain and immune status of the animal. Clinically severe BVDV, also known as severe acute BVDV (SA BVDV), occurs in animals infected with a type 2 BVDV that have no or low titers against BVDV2 strains. It is associated with a greater than 50% reduction in circulating lymphocytes and platelets and body temperatures exceeding 40.6 °C (Carman et al., 1998; Liebler-Tenorio et al., 2002, 2003b). Severe acute BVD may progress to hemorrhagic syndrome in some cases (Corapi et al., 1990, 1989; Stoffregen et al., 2000). Viral antigen may be detected in epithelial cells following infection in vivo, however, the principle site of replication and spread of the virus is in lymphoid tissue (Liebler-Tenorio et al., 2002, 2003a,b). While both clinically severe and subclinical BVDV infections are associated with lymphoid depletion, the depletion seen with highly virulent strains is more extensive and longer in duration (Liebler-Tenorio et al., 2002, 2003a,b). Although lymphoid tissue is a major replication site of the virus in vivo and pathogenesis is associated with depletion of lymphoid tissues, most in vitro studies have been done using cultured epithelial cells because of the greater availability of epithelial cell lines.

Initial studies detailed here compared the in vivo effects of infection following infection with two type 2 noncytopathogenic BVDV with different levels of expressed virulence. While infection with one virus, BVDV2-RS866, resulted in subclinical or mild clinical signs, infection with the other virus, BVDV2-1373, resulted in severe clinical disease. We attempted to develop an in vitro model to study the differences in virulence displayed by these two viruses. To this end, we compared the results of infection on cultured epithelial and lymphoid cells lines in vitro. While we saw no difference between the two viruses in cultured epithelial cells, we observed a decrease in replicating cells in cultured lymphoid cell lines. To determine if this cell death was similar to the cytopathology observed with cytopathic BVDV strains, we included a cytopathic strain from the same genotype, BVDV2-296c, in the cultured lymphocyte studies. While infection with both BVDV2-1373 and BVDV2-296c resulted in cell death in BL-3 cultures, the time lapse between infection and cell death and the pathway involved in cell death were different.

2. Materials and methods

2.1. Isolation, characterization and propagation of viruses

The BVDV2 strains used in this study originated in the United States or Canada and were isolated between 1993 and 1998. Strain BVDV2-1373 was isolated from a severe acute BVDV outbreak in Ont., Canada (Carman et al., 1998). This strain reproducibly causes severe acute disease in calves seronegative to BVDV (Liebler-Tenorio et al., 2002; Stoffregen et al., 2000). The noncytopathic strains BVDV2-28508-5 and BVDV2-RS886 were isolated from persistently infected asymptomatic calves. Infection with either of these strains results in a subclinical infection (Liebler-Tenorio et al., 2003a, 2004; Ridpath et al., 2000). The cytopathic/noncytopathic pair BVDV2-296c and BVDV2-296nc was isolated from a mucosal disease case (Ridpath and Neill, 2000). Strains were assigned to BVDV genotype 2 based on phylogenetic analysis of the 5′ UTR region (Ridpath et al., 1994). Strains were assigned to the cytopathogenic or noncytopathogenic biotype based on activity in cultured bovine epithelial cells (Gillespie et al., 1960) and production of NS3 (Donis and Dubovi, 1987; Pocock et al., 1987) as determined by radioimmunoprecipitation using bovine polyclonal antisera (Ridpath and Bolin, 1990) as described in previous publications (Liebler-Tenorio et al., 2003a, 2004; Ridpath et al., 1994, 2000). Viruses were propagated as described earlier (Ridpath et al., 2002) with the exception that the Madin Darby bovine kidney (MDBK) cell line was used rather than bovine turbinate cells. Fetal bovine serum used to supplement cell culture medium was tested free of BVDV and antibodies against BVDV (Bolin et al., 1991b).

2.2. Animal model

Mixed breed calves were caught at birth and fed milk replacer that was tested free of BVDV and antibodies to BVDV. All calves tested negative for BVDV at birth, as determined by virus isolation from buffy coat samples followed by detection based on polymerase chain reaction (PCR) assay and immunohistochemistry staining for the presence of BVDV antigens in skin (Ridpath et al., 2002). Virus isolation from buffy coat samples was also performed on samples collected immediately preceding virus inoculation to assure that animals were free of circulating BVDV at the time of inoculation. In addition, calves were tested free of antibodies against BVDV at birth and immediately before inoculation with virus as determined by serum neutralization using BVDV type-1 strain BVDV1-NY-1 and BVDV type-2 strain BVDV2-1373 (Ridpath et al., 2002).

Age at inoculation ranged from 2 to 9 months. Nine calves were infected with BVDV2-1373 and five were infected with BVDV2-RS886. Four calves served as noninfected controls. Animals were infected with 5 ml of inoculum [titer of $1 \times 10^6\ ml^{-1}$ tissue culture infectious dose (TCID)] by the oral/nasal route. Temperatures were taken daily. Blood samples for determination of white blood cell (WBC) counts were collected on days 0, 2, 4, 6, 9, 11 and 13 post inoculation. The WBC counts were determined using an HV 1500 cytometer (CDC Technologies, Inc., Oxford, CT) per manufacturer's directions. Buffy coat samples were collected on days 0, 3 or 4, 9 and 13 days post infection.

Blood was collected in buffered sodium citrate on days 0, 2, 4, 6, 9, 11 and 13 days post infection for flow cytometric analysis of dead (propidium iodide uptake) and apoptotic (annexin binding) circulating WBC. Propidium iodide uptake and annexin binding were done using a TACS Annexin V kit (Trevigen, Inc., Gaithersburg, MD) and processed for flow cytometry per manufacturer's

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