A Microtiter Test for Detecting and Titrating Noncytopathogenic Bovine Viral Diarrhea Virus

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Summary

Bovine cells free of noncytopathogenic bovine viral diarrhea virus (NC-BVDV) treated with polyriboinosinic acid : polyriboctydylidic acid (polyI:C) were protected against challenge with vesicular stomatitis virus (VSV), whereas NC-BVDV-infected cells treated with polyI:C were not protected against VSV. An assay based on the ability of NC-BVDV to inhibit polyI:C protection of cells against VSV was developed and is herein referred to as PINBA (polyI:C for NC-BVDV assay).

Noncytopathogenic BVDV was titrated as cytopathogenic strains except that several days after infection with NC-BVDV, the cultures were treated with polyI:C and VSV. Titration endpoints were reached 24 hours later. PINBA was standardized for amount of VSV, time of addition of polyI:C, and time NC-BVDV had to be present to obtain stable titration endpoints. PINBA also was useful for titrating virus neutralizing antibodies. Compared with the fluorescent antibody test, PINBA was less subjective for detection of NC-BVDV. Compared with the interference test in which NC-BVDV infected cultures are challenged with a cytopathogenic strain of BVDV, PINBA was more reliable.

The technique described herein is a simple and practical microtiter method for titrating NC-BVDV and virus neutralizing antibodies and for the presumptive detection of NC-BVDV.

Introduction

Bovine viral diarrhea virus (BVDV), a common pathogen of cattle, often causes subclinical as well as clinical infections and induces high and persistent virus neutralizing (VN) serum antibody titers (11, 20). The virus can produce a chronic infection that may suppress the immune system and thereby enhance disease due to other agents (11, 13, 19). BVDV crosses the placenta (1, 3, 27) and is frequently isolated from commercial fetal bovine serum (FBS) (15, 16, 23, 28), aborted and normal fetuses (19), and tissues cultured from bovine embryos (2, 9,
Most isolates of BVDV from clinical cases as well as from bovine fetal and embryo products have been noncytopathogenic (NC-BVDV) (19, 20). BVDV and hog cholera virus (HCV) have the capacity to enhance the replication and cytopathogenicity of Newcastle disease virus (NDV) in vitro (12, 17, 18). The enhancement of NDV cytopathogenicity by BVDV or HCV is the basis for diagnostic tests for NC-BVDV [bovine exaltation of NDV (BEND)] (12) and HCV [exaltation of NDV (END)] (17, 18). Dieterholm and Dinter (4) have reported that BVDV inhibits the activity of NDV-induced IFN so that NDV can replicate to high titers and produce a marked cytopathic effect (CPE). Rossi and Kiesel (24) subsequently confirmed that NC-BVDV-infected cultures prevented the antiviral effect of polyriboinosinic acid-polyribocytidylic acid (poly I:C) induced IFN, and showed that BVDV infected cultures did not interfere with induction of IFN by poly I:C (25).

Presently available techniques for titrating NC-BVDV include BEND (12), the interference of NC-BVDV for cytopathogenic BVDV (8), and fluorescent antibody (FA) tests (5). These tests have not found wide acceptance. Results obtained with the interference test are often unreliable due to the instability of endpoints. Use of the FA test to titrate NC-BVDV requires an almost prohibitive amount of work. The purpose of the present investigation was to develop a simple microtiter test for detecting and titrating NC-BVDV based on observations that cells treated with poly I:C and challenged with vesicular stomatitis virus (VSV) were protected against VSV, whereas NC-BVDV infected-cells treated with poly I:C were not protected against VSV. The test that has been developed will be referred to as PINBA (poly I:C for NC-BVDV assay).

**Materials and Methods**

**Tissue Cultures**

Cell cultures free of NC-BVDV derived from bovine embryonic lung and kidney, whole bovine embryo, and bovine testicles and permanent bovine cell lines AU-BEK, GBK, and MDBK that were persistently infected with NC-BVDV were used. Unless otherwise indicated, bovine embryonic lung cells were used. Growth medium consisted of Eagle's minimum essential medium (EMEM) with 10 percent fetal bovine serum (FBS), 100 U of penicillin/ml, 50 µg of neomycin/ml and 200 µg of streptomycin/ml. When the medium was used for microtiter plates, 1 percent of 1 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 0.75 percent of 1 M tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), and 0.75 percent of 1 M morpholino propane sulfonic acid (MOPS) were added. Cells and FBS were pretested by the FA test to be certain they were free of BVDV (23).

**Viruses**

NC-BVDV-E3289 isolated from commercial FBS was used for most of the titration studies. Fifteen other NC-BVDV isolates from FBS or from permanent bovine cell lines, and the Sanders strain of BVDV were used to determine the efficacy of PINBA for detecting NC-BVDV. All the NC-BVDV isolated from FBS had been originally identified by the FA test. Cytopathogenic BVDV-NADL was cloned by terminal dilution and there was no NC-BVDV, as determined by PINBA, beyond the cytopathic endpoint in titrations of BVDV-NADL. The New Jersey serotype of vesicular stomatitis virus (VSV) was used at 1000 TCID<sub>50</sub> as challenge virus unless otherwise indicated.