Detection of Rat, Porcine, and Bovine Group B Rotavirus in Fecal Specimens by Solid-Phase Enzyme Immunoassay

STEVEN L. VONDERFECHT,1,* DAVID A. LINDSAY,2 AND JOSEPH J. EIDEN3

Drug Safety Research, The Upjohn Company, Kalamazoo, Michigan 49001;1 Department of Chemical Engineering, The Johns Hopkins University, Baltimore, Maryland 212182; and Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 212053

Received 21 October 1993/Returned for modification 24 November 1993/Accepted 23 December 1993

An enzyme immunoassay that uses easily regenerated reagents was developed and evaluated for the ability to detect group B rotaviruses (GBR) in fecal specimens. Homologous rat GBR and heterologous porcine and bovine GBR were detected by this immunoassay, although a human GBR isolate was not. This immunoassay should prove useful in studies of GBR infections of animals.

The group B rotaviruses (GBR) are morphologically identical to but antigenically and genetically distinct from the typical or group A rotaviruses (GAR). GBR have been shown to be a cause of diarrhea in pigs, calves, lambs, rats, and humans (3, 10); however, the prevalence and significance of these viruses in any species have yet to be firmly established. This is at least partially due to the difficulties in obtaining GBR reagents that are well characterized, easily regenerated, and widely available. Additionally, GBR have not been adapted to cell cultures, making it necessary to derive viral preparations from infected fecal material. This has made the preparation of high-quality hyperimmune serum suitable for use in immunoassays difficult. One of the goals of our laboratories has been to develop well-characterized, easily renewable reagents that could be used in a sensitive and specific immunoassay for the detection of GBR infections. In this report, we describe the use of such reagents to develop a solid-phase enzyme immunoassay for the detection of a variety of animal GBR.

The immunoassay evaluated was a "sandwich-type," solid-phase enzyme immunoassay in which a mouse monoclonal antibody to the infectious diarrhea of infant rats (IDIR) virus (12) was used to coat the solid phase and a rabbit antibody to IDIR virus was used as the secondary or detecting antibody.

The development and characterization of anti-IDIR virus monoclonal antibody 15-S3 has been reported previously (14). This antibody recognizes the major inner capsid protein of IDIR virus which is encoded by the sixth segment of the viral genome (13). The rabbit antibody to IDIR virus was produced by immunizing rabbits with lysates obtained from Sf-9 insect cells infected with a recombinant baculovirus containing the sixth segment of the IDIR virus genome. The construction of the baculovirus recombinant, production of the antibody preparation, and characterization of the IDIR virus reactivity of the antibody preparation have been described previously (8). The polyclonal rabbit antibody, like the mouse monoclonal antibody, is monospecific and recognizes the major inner capsid protein of IDIR virus. The control rabbit antibody used in the enzyme immunoassay was obtained from a rabbit immunized with lysates of Sf-9 cells infected with a baculovirus recombinant containing IDIR virus gene 8. Antibodies to IDIR virus were not found in the control serum or in preimmunization sera by Western immunoblot, immunofluorescence, or enzyme immunoassay methods.

In order to conduct the enzyme immunoassay for GBR antigens, the wells of polystyrene microtiter plates were first coated with monoclonal antibody 15-S3 produced in mouse ascites. Following the addition of monoclonal antibody, test fecal specimen, control rabbit serum or rabbit antibody to the IDIR virus gene 6 protein, peroxidase-labeled goat antibody to rabbit immunoglobulin G, and the substrate TMB (3,3′,5′,5′-tetramethylbenzidine) were added. The reaction of the enzyme on the substrate was stopped after approximately 10 min by the addition of 1 M phosphoric acid, and the optical density (OD) of the reaction mixture was determined in a microplate colorimeter at a wavelength of 450 nm. The specific activity of a particular sample was calculated by subtracting the ODs of the wells covered with the control rabbit antibody from the ODs of the wells covered with the rabbit antibody to IDIR virus.

The immunoassay was quite sensitive at detecting homologous IDIR virus. A sample of pooled intestinal washings collected from IDIR virus-infected suckling rats was diluted 1:3,200 and still produced a specific activity of more than 1.0 OD units. In contrast, the control specimen of pooled intestinal washings, used at the same dilution, had a specific activity of zero. In addition to detecting the homologous virus, the immunoassay was also used to detect the heterologous GBR of pigs and calves. Fecal specimens collected from two piglets experimentally infected with a porcine strain of GBR tested positive, while fecal specimens obtained from healthy, GAR-infected, or group C rotavirus-infected suckling pigs had minimal activity. Similarly, diarrheic feces from three calves inoculated with the bovine enteric syncytial virus strain of GBR each tested positive. Fecal specimens from healthy and GAR-infected suckling calves were negative. The results of the enzyme immunoassay for detection of heterologous group B rotaviruses in porcine and bovine fecal specimens were as follows (results are given for specimens tested at a 1/400 dilution; +, net OD reading of ≥0.5; −; net OD reading of <0.05): porcine GBR-1, +; porcine GBR-2, +; porcine GAR, −; porcine group C rotavirus, −; healthy pig, −; bovine GBR-1, +; bovine GBR-2, +; bovine GBR-3, +; bovine GAR, −; healthy cow 1, −; and healthy cow 2, −.

In contrast to our ability to detect heterologous animal strains of GBR by the immunoassay, we were unable to detect an adult diarrhea rotavirus (ADR/V) strain of GBR from humans. The reasons for this are not entirely clear. A previous

* Corresponding author. Mailing address: The Upjohn Company, 7220-300-1, 301 Henrietta St., Kalamazoo, MI 49001. Phone: (616) 385-7823. Fax: (616) 384-9331.
study indicates that anti-IDIR virus monoclonal antibody 15-S3 reacts with a 47-kDa protein of ADRV, which is considered to be the major inner capsid protein of the virus (5). Although we found that the rabbit antibody to the baculovirus-expressed IDIR virus gene 6 product reacted in an enzyme immunoassay with the particular isolate of ADRV used in the present study, we were unable to demonstrate that monoclonal antibody 15-S3 recognized this isolate in either enzyme immunoassay or Western immunoblot formats (data not shown). The relationship between the isolate of ADRV used in the previous study and the viral specimen used in the present study is not known. Studies with GAR have documented the presence of subgroup antigens that are located on the major inner capsid protein (7). Most of the animal GAR are subgroup I, while many, but not all, of the human GAR are subgroup II. Recent investigations with isolates of human GBR have suggested that differences may exist among the isolates in the gene-to-protein-coding assignment for the major inner capsid antigen (6). GAR-like subgroup antigens or other antigenic differences in the major inner capsid protein of GBR could account for the disparate reactivities between the human and animal strains of GBR in the immunoassay. The identification of such antigenic differences should be the subject of further investigation.

The GBR antigen detection immunoassay described in this report used reagents that can be produced by relatively simple laboratory techniques. Previous studies performed to detect the presence of GBR in fecal specimens or to determine the prevalence of GBR infections have used reagents that are of limited supply and uncharacterized reactivity (1, 2, 4, 9, 11). These limitations make comparisons among individual studies difficult. Our ability to construct an enzyme immunoassay with a monoclonal antibody and an antibody against a GBR protein expressed by a baculovirus recombinant obviates these limitations. The failure to detect the human strain of GBR by the immunoassay would preclude its use in studies to determine the epidemiology of human GBR. However, these reagents should prove useful in future studies in animals designed to examine the incidence, diagnosis, and replication kinetics of GBR.

We acknowledge Kenneth Theil (Ohio Agricultural Research and Development Center, Wooster), Alfonso Torres (College of Veterinary Medicine, Cornell University, Ithaca, N.Y.), and Roger Glass (Viral Gastroenteritis Unit, Centers for Disease Control and Prevention, Atlanta, Ga.) for the contribution of porcine, bovine, and human fecal specimens, respectively.

This work was supported in part by NIH grant R29 AI24922 (to J. J. Eiden).

REFERENCES