In Vitro Detection of Porcine Rotavirus-Like Virus (Group B Rotavirus) and Its Antibody†

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Four isolates of porcine rotavirus-like virus (PRVLV) infected MA104 cells and induced syncytium formation after low-speed centrifugation of the inoculum onto the monolayer. Ten of 44 (23%) Ohio swine sera had PRVLV antibodies when tested by indirect immunofluorescence, using PRVLV-infected MA104 cell monolayers as antigen.

Early studies established that porcine rotaviruses shared a common group antigen with rotaviruses isolated from other host species (3, 19). Recently, however, two antigenically distinct and unrelated atypical rotaviruses (rotavirus-like agent and pararotaviruses) were also isolated from young pigs (4, 6, 12, 14). These findings prompted the proposal that rotaviruses be subdivided into groups, with members of each group sharing their own distinctive common antigen (12). Accordingly, the original rotaviruses would comprise group A, whereas the rotavirus-like agent and the pararotavirus would belong to groups B and C, respectively.

Additional atypical rotaviruses have now been recovered from other mammalian species, including humans (8, 9, 11, 13, 15, 16, 18). Antigenic comparisons of atypical rotaviruses remain difficult, and such studies are usually limited to laboratories with gnotobiotic animals (4, 12, 15), as suitable cell culture systems are often lacking. Infected cell cultures would provide a convenient source of viral antigen useful for antigenic comparisons among these viruses and for serological surveys to determine whether infections with atypical rotaviruses are prevalent.

We recently described a porcine rotavirus-like virus (PRVLV) that was antigenically unrelated to the group A and the group C rotaviruses, and we have tentatively considered it a group B rotavirus (17). This virus did not adapt to serial passage in cell cultures under procedures commonly employed for group A rotavirus propagation. This report describes a simple method, similar to one originally used with rotaviruses (2, 7), of infecting MA104 cell monolayers with PRVLV by centrifugation of the virus inoculum onto the monolayer.

Monolayers were grown to confluency in 8-well plates (Miles Laboratories, Inc., Naperville, Ill.) containing glass cover slips (24 by 30 mm), using Eagle minimum essential medium prepared in Earle balanced salt solution (EMEM) supplemented with 100 U of penicillin per ml, 100 µg of streptomycin per ml, 25 U of mycostatin per ml, and 10% fetal bovine serum. Before inoculation, growth medium was removed and monolayers were washed three times with serum-free EMEM. Specimens for inoculation were intestinal contents of infected gnotobiotic pigs diluted 25-fold in serum-free EMEM and clarified by low-speed centrifugation. Approximately 1 ml of inoculum was added per well

and then centrifuged onto the monolayer at 400 to 660 × g for 1 h at ambient temperature, using microtiter plate centrifuge carriers (Dynatech Laboratories, Inc., Alexandria, Va.). Monolayers treated similarly with 1 ml of serum-free EMEM served as uninoculated controls. After centrifugation, the inoculum was removed and the monolayers were incubated for 16 to 20 h with serum-free EMEM at 37°C in a 5% CO₂ atmosphere. Cover slip monolayers were fixed with acetone, and PRVLV-infected cells were detected by an indirect immunofluorescent stain, using hyperimmune gnotobiotic pig serum (17); this hyperimmune serum did not react with porcine rotavirus, porcine pararotavirus, or reovirus type 3 antigens in indirect immunofluorescent stains. Additional cover slip monolayers fixed with absolute methanol were stained by the May-Grunwald Giemsa method (10).

By this procedure, the Ohio isolate of PRVLV consistently produced a specific cytopathic effect that was readily discernable in unstained MA104 cell monolayers at low magnification (40×) and consisted of the formation of multinucleated giant cells (syncytia). The smaller syncytia usually contained 50 to 100 nuclei, some of which were swollen, within a common cytoplasmic mass; cytoplasmic inclusion bodies were not observed (Fig. 1). Larger syncytia contained hundreds of nuclei. The periphery of a large syncytium frequently contained many large nuclei arranged in a ring, whereas the center contained numerous pyknotic nuclei surrounded by vacuolated cytoplasm. These syncytia did not enlarge with prolonged incubation (72 to 96 h) but often detached, leaving holes within the monolayer. Three additional antigenically related PRVLV isolates (SC, KH-822, and N-338 isolates) also induced syncytium formation in MA104 cell monolayers when inoculated by this procedure. At present, the sensitivity of this assay for PRVLV appears low: most specimens from infected gnotobiotic pigs that were infective for MA104 cells contained only 10 to 50 syncytium forming units per ml at a 1:25 dilution. Syncytia were never observed in the control monolayers or in monolayers similarly inoculated with porcine calicivirus-like virus (14), rotavirus, pararotavirus, or transmissible gastroenteritis virus.

Attempts to serially passage PRVLV in MA104 cells by using this centrifugation procedure and cell culture fluids derived from monolayers containing syncytia proved unsuccessful, as syncytia could not be detected in inoculated monolayers after the second passage. Apparently, there are

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† Journal article no. 211-84 from the Ohio Agriculture Research and Development Center, The Ohio State University.

FIG. 1. Syncytium formation in MA104 cells induced by PRVLV (Ohio isolate). (A) Noninfected control monolayer, 20 h after centrifugation. (B and C) Infected monolayers, 20 h after centrifugation, containing syncytia. May-Grunwald Giemsa stain. Magnification, 100 $\times$.

few, if any, virions produced within these syncytia that are readily infective for other MA104 cells.

Syncytia contained large quantities of PRVLV antigen, as indicated by their intense intracytoplasmic immunofluorescence (Fig. 2). Positive immunofluorescent reactions were also detected with syncytia stained by the indirect method with hyperimmune anti-bovine RVLV serum (17), but were less intense than that obtained with the homologous hyperimmune anti-PRVLV serum. This agreed with our previous findings (17) and demonstrated that these syncytia are a

suitable alternative to infected small intestine tissue as an antigen source for comparative studies.

Vero and CV-1 cells were less susceptible to infection with PRVLV than were MA104 cells. Monolayers of these cell lines were inoculated as described above with seven specimens previously shown to induce syncytium formation in MA104 cell monolayers. Immunofluorescent staining revealed that only one of these specimens was infective for CV-1 cells (inducing very small syncytia) and none was infective for the Vero cells.

An in vitro source of PRVLV antigen can facilitate preliminary serological surveys to establish the prevalence of PRVLV antibodies. Forty-four sera from swine in three Ohio herds (Table 1) were tested at a 1:10 dilution by indirect immunofluorescence for PRVLV antibodies, using MA104 cell monolayers infected with PRVLV (Ohio isolate). Dilutions of sera from two PRVLV hyperimmune gnotobiotic pigs were also tested. Monolayers in 96-well plates were inoculated by centrifugation as before, except that 0.2 ml of inoculum was added per well. After overnight incubation, monolayers containing syncytia were fixed in 80% acetone and used for antibody testing. All sera were also tested at a

FIG. 2. Indirect immunofluorescent staining of MA104 cell monolayers, using gnotobiotic pig hyperimmune antiporcine rotavirus-like virus serum. (A) Noninfected control monolayer 18 h after centrifugation. (B) Monolayer infected with PRVLV (Ohio isolate), 18 h after centrifugation. Note syncytium with intense intracytoplasmic immunofluorescence. Magnification, 80 $\times$.

TABLE 1. Prevalence of PRVLV and rotavirus antibodies in swine sera as determined by an indirect immunofluorescence test

Sera from:	Age of pigs	No. tested	No. positive for antibody to:	
			Rotavirus	PRVLV
Herd A	10 mo	36	31	9
Herd B	8 wk	5	5	0
	Adult	1	1	0
Herd C	3 wk	2	2	1
PRVLV-hyperimmune ^a	8 wk	2	0	2

^a Prepared in gnotobiotic pigs.

1:10 dilution for rotavirus antibodies, using MA104 cells infected with porcine rotavirus (OSU isolate). Both PRVLV hyperimmune sera gave intensely positive immunofluorescence reactions with PRVLV syncytia when tested at a 1:240 dilution but were seronegative for rotavirus antibodies at a 1:10 dilution. Swine seropositive for PRVLV antibodies were found in two of three herds, but the proportion positive (23%) was considerably less than the proportion positive for rotavirus antibody (89%). The percentage of swine seropositive for rotavirus antibodies agreed closely with our previous serological survey (5). Although it is impossible to precisely standardize the sensitivities of these two immunofluorescence assays, the relatively low percentage of swine seropositive for PRVLV antibody suggests that infections with this virus are likely to have a pattern different from that of rotavirus infections or that PRVLV infections fail to induce high serum antibody levels.

Although syncytia occur within the small intestinal mucosa of rats and pigs infected with atypical rotaviruses (1, 18), this is the first report of an atypical rotavirus inducing syncytium formation in cell culture. Although this cell culture assay for PRVLV does appear to be somewhat insensitive, it nonetheless represents the only *in vitro* assay for detecting infective PRVLV in specimens and also provides a useful alternative source of PRVLV antigen for serological tests. Efforts to improve the sensitivity of this assay are under way.

This research was supported in part by Public Health Service research grant AI-21621-01 from the National Institute of Allergy and Infectious Diseases and by Special Grants Program no. 83-CRSR-2-2286, U.S. Department of Agriculture Science and Education Administration, Cooperative State Research Service. Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

We thank Arden Agnes, Richard Braun, Mary Beth Kaps, Christine McCloskey, and Glen Burkey for technical assistance.

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