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Antigenic Comparisons of Two New Rotaviruses from Rhesus Monkeys

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Two rotavirus strains isolated in cell culture from infant rhesus monkeys with diarrhea were closely related to SA 11 virus and to each other by plaque reduction neutralization tests. However, results of immune electron microscopy suggested possible antigenic differences between the two rhesus rotavirus strains.

The simian rotavirus SA 11 was isolated from an asymptomatic vervet monkey more than 20 years ago (2). It has been widely used as a source of antigen for detecting human rotavirus antibody, since it grows to high titer in cell culture whereas human rotaviruses do not. In a recent study on viral diarrhea in monkeys (5), two new strains of simian rotavirus, propagable in cell culture, were isolated from infant rhesus monkeys with diarrhea. These were tentatively designated S:USA:78:1 and S:USA:79:2, in accord with the nomenclature proposed by G. N. Woode (personal communication). The monkeys were born and raised at the California Primate Research Center, University of California, Davis. This report describes antigenic comparisons of these rhesus rotaviruses with SA 11 virus performed by plaque reduction neutralization tests and by immune electron microscopy (IEM).

The S:USA:78:1 strain was isolated from a stool sample collected 1 day after the onset of diarrhea in a 4-month-old animal designated MMU 17959. The specimen produced cytopathic effects characteristic of both adenovirus and rotavirus in primary cynomolgus monkey kidney (CMK) cell cultures, involving 50% of the cells after 4 days of incubation at 36°C. Electron microscopy on the cell culture material revealed a mixture of adenovirus and typical rotavirus particles. The adenovirus was identified as SV 17. Rotavirus was separated from the adenovirus by four serial passages in primary CMK cells in the presence of 60 μg of iododeoxyuridine per ml of medium, followed by two plaque purifications in a line of fetal monkey kidney cells (4). The rotavirus was then propagated in primary CMK cells, attaining a titer of $10^{4.5}$ plaque-forming units per ml. A serum specimen taken from the monkey 36 days after the onset of diarrhea had a rotavirus antibody titer of 1:1,024 in an enzyme immunoassay using SA 11 virus as a source of antigen (6). An acute-phase serum was not available.

The S:USA:79:2 strain was isolated from a stool sample collected 1 day after the onset of diarrhea in a 3.5-month-old monkey designated MMU 18006. In primary CMK cells the specimen produced a cytopathic effect characteristic of rotavirus in 25% of the cells after 9 days incubation at 36°C. IEM on the cell culture material showed a pure preparation of typical rotavirus particles. This isolate was carried for six serial passages in primary CMK, plaque-purified twice in fetal monkey kidney cells, and then passed again into primary CMK cells, reaching a titer of $10^{5.5}$ plaque-forming units per ml. A serum specimen collected 13 days before onset of diarrhea had a rotavirus enzyme immunoassay antibody titer of <1:32, whereas sera collected at 25 and 50 days after onset had titers of 1:256 and 1:512, respectively. A hyperimmune serum to this strain was also prepared in rhesus monkeys.

Plaque reduction neutralization tests were performed by a minor modification of the method of Matsuno et al. (3), using BS-C-1 cells grown in no. 3524 tissue culture clusters (Costar, Cambridge, Mass.). The addition of diethylaminoethyl-dextran to the first overlay was omitted. A test dose of 50 plaque-forming units of virus was used with serial twofold dilutions of sera. The antisera were a SA 11 hyperimmune serum produced in a rhesus monkey and the convalescent-phase sera of the rhesus monkeys from which rotavirus was isolated. Pre-immunization serum from the SA 11 immune monkey was used as a control. Serum-virus mixtures were incubated at 4°C for 1 h before inoculation onto the cell cultures. Antibody endpoints were expressed as the highest initial serum dilution producing a 50% or greater reduction in plaque count, as compared with controls consisting of the test virus mixed with pre-immunization serum or with diluent. The diameter of plaques was 0.5 to 1.0 mm for the SA 11 and S:USA:78:1

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TABLE 1. *Results of cross-plaque reduction neutralization tests with SA 11 and rhesus rotavirus strains*

Virus	Neutralizing antibody titers of antiserum to[a]:		
	SA 11	S:USA:78:1	S:USA:79:2
SA 11	65,536	128	32
S:USA:78:1	16,384	128	16
S:USA:79:2	16,384	64	32

[a] Each value represents reciprocal of antibody titer.

strains and 1.5 to 2.0 mm for the S:USA:79:2 strain. Results of plaque reduction neutralization tests are shown in Table 1. The titer of the SA 11 antiserum was fourfold higher for the homologous virus than for the two heterologous strains. Antisera to the rhesus rotavirus strains had homologous and heterologous titers which were identical or differed by no more than twofold.

IEM was performed by the method described by Kapikian et al. (1), using infected cell culture fluids. In addition, the original stool suspension was used as antigen for the S:USA:78:1 strain. A 10% fecal suspension prepared in phosphate-buffered saline by shaking with glass beads was clarified by centrifugation at 10,000 × *g* for 20 min. A 0.9-ml volume of antigen was mixed with 0.1 ml of a 1:10 dilution of serum and incubated at 37°C for 1 h followed by 4°C overnight. After centrifugation at 35,000 × *g* for 60 min, the resulting pellet was suspended in 1 or 2 drops of distilled water. The suspension was mounted on a grid, and the excess fluid was blotted off before staining with 3% phosphotungstic acid, pH 7.0, and examining by IEM for virus-antibody complexes.

Results of cross IEM tests are as follows. SA 11 virus reacted with homologous and heterologous antisera in similar fashion; immune complexes contained 50 to 80 viral particles, both single- and double-shelled, and very few individual particles were observed. The S:USA:78:1 cell culture preparation and stool suspension reacted similarly with homologous antiserum and with SA 11 antiserum; large immune complexes contained both single- and double-shelled particles, and individual (unagglutinated) viral particles were rarely seen. However, when S:USA:78:1 stool and cell culture preparations were tested against the S:USA:79:2 convalescent and hyperimmune sera, virus-antibody complexes were fewer and contained only five to six viral particles; large numbers of unagglutinated single- and double-shelled particles were seen. The S:USA:79:2 stool suspension contained too few viral particles to permit an accurate antigenic analysis by IEM. However, S:USA:79:2 virus particles in a cell culture preparation showed less clumping (i.e., few small aggregates of single- and double-shelled particles, and large numbers of unagglutinated particles) with the heterologous S:USA:78:1 antiserum than with the homologous antisera. S:USA:79:2 cell culture virus reacted with SA 11 antiserum, as well as with S:USA:79:2 convalescent and hyperimmune serum, to give large aggregates of single- and double-shelled particles and few individual, unagglutinated viral particles.

The rotaviruses from macaque and vervet monkeys are similar in their ability to grow in cell culture, and results of both cross-neutralization and IEM tests indicated a close antigenic relationship of the rhesus viruses to SA 11 virus. The animals from which the viruses were isolated had no contact with vervet monkeys or with animal handlers who cared for vervet monkeys. Whether the two new rhesus strains will be as useful as the SA 11 strain as a source of antigen for human rotavirus antibody assays remains to be established. Although cross-neutralization tests did not reveal differences between the two rhesus rotavirus strains which could be considered significant, cross-testing by IEM suggested possible antigenic differences. The two strains also differed in the size of plaques which they produced. Possibly monoclonal antibodies or polyacrylamide gel electrophoresis of viral ribonucleic acid will reveal more clear-cut differences between the two rhesus rotavirus strains.

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