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Serological Comparison of Canine Rotavirus with Various Simian and Human Rotaviruses by Plaque Reduction Neutralization and Hemagglutination Inhibition Tests

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By the plaque reduction neutralization test, the CU-1 strain of canine rotavirus was similar, if not identical, to three strains (no. 14, no. 15, and P) of the tentatively designated third human rotavirus serotype. In addition, strain CU-1 demonstrated a one-way antigenic relationship with two other strains (M and B) of the third human rotavirus serotype. The CU-1 strain of canine rotavirus hemagglutinated human group O, rhesus monkey, dog, sheep, and guinea pig erythrocytes. A two-way antigenic relationship between canine (CU-1) and simian (MMU 18006 and SA11) rotaviruses demonstrated previously by the plaque reduction neutralization test was confirmed further with two additional isolates (A79-10 and LSU 79C-36) of canine rotavirus by the plaque reduction neutralization test and the hemagglutination inhibition test. The CU-1 strain of canine rotavirus, which is known to be distinct from two well-characterized human rotavirus serotypes (Wa and DS-1), was also found to be distinct from the St. Thomas no. 4 strain, which is a newly defined fourth human rotavirus serotype. Thus, this canine strain, which is related antigenically to one of four human rotavirus serotypes, is another example of an animal rotavirus which shares serotype specificity with a human rotavirus.

In 1981, Kapikian et al. (10) reported that some human and animal rotaviruses are in the same realm of a classification scheme by demonstrating that certain animal rotaviruses such as NCDV and UK share an antigenic specificity (designated subgroup) with human rotavirus DS-1, but not with human rotavirus Wa, by immune adherence hemagglutination assay and enzyme-linked immunosorbent assay. Moreover, Kalica et al. demonstrated that neutralization and subgroup antigens are coded for by different genes (8). Wyatt et al. (14) reported in 1982 that both human and animal rotaviruses are in the same realm of a serotyping scheme by demonstrating that simian rotaviruses (strains MMU 18006 and SA11) belong to the tentatively designated third human rotavirus serotype. We reported previously, for the first time, an example of a rotavirus serotype common to two animal species; a canine rotavirus (CU-1) is similar, if not identical, to simian rotaviruses (MMU 18006 and SA11) by the plaque reduction neutralization (PRN) test (7). The purpose of the present paper is to describe the further characterization of the canine rotavirus (CU-1), including antigenic relationships of this agent with two other isolates of canine rotavirus (strains A79-10 and LSU 79C-36) and with newly defined third (14) and fourth (15) human rotavirus serotypes.

MATERIALS AND METHODS

Cell cultures. An established cell line of fetal rhesus monkey kidney, MA 104, was used for virus propagation, titration, and PRN assay. Cells were grown in plastic six-well plates (Costar, Cambridge, Mass.) by M. A. Bioproducts, Walkersville, Md., or in our laboratory.

Viruses. The CU-1 strain of canine rotavirus was isolated at the Cornell Feline Health Center, Ithaca, N.Y. (7). The A79-10 strain of canine rotavirus was supplied by M. J. Appel; the LSU 79C-36 strain of canine rotavirus was supplied by G. N. Woode; the NCDV strain of bovine rotavirus was supplied by C. A. Mebus; the OSU strain of porcine rotavirus was supplied by E. H. Bohl; the SA11 strain of simian rotavirus was supplied by H. Malherbe; the MMU 18006 strain of simian rotavirus was supplied by N. J. Schmidt. Human rotavirus strains DS-1, M, B, no. 14, no. 15, and St. Thomas no. 4 are reassortant viruses produced by coinfection with temperature-sensitive mutants of cultivatable bovine rotavirus (UK) and noncultivatable human rotaviruses (5, 6). All rotaviruses were plaque purified before use.

Hyperimmune antisera. All hyperimmune antisera

were prepared in guinea pigs. Procedures for the preparation of hyperimmune antisera were described previously (5, 14).

PRN test. The PRN test was performed as described previously (7, 14). The neutralizing titer was expressed as the reciprocal of the calculated serum dilution which caused a 60% reduction in plaque count. If the difference between homologous and heterologous neutralizing titers was 20-fold or greater, viruses were considered to be significantly different and thus serotypically distinct; if the difference between the titers was less than 20-fold, the viruses were considered to be antigenically related and thus not serotypically distinct.

Subgroup antigen assay. Either the immune adherence hemagglutination assay (10) or the enzyme-linked immunosorbent assay (4) or both were employed to subgroup canine rotaviruses (CU-1, A79-10, and LSU 79C-36).

Hemagglutination and HI tests. The infected cell lysates were extracted three times with Genetron 113, followed by sedimentation through a 40% sucrose cushion by ultracentrifugation. The pellets were suspended with phosphate-buffered saline to give 1/30 of the original volume of the cell lysates and used for hemagglutination and hemagglutination inhibition (HI) tests. The human group O, dog, rhesus monkey, sheep, and guinea pig erythrocytes were used at a 0.5% concentration in phosphate-buffered saline supplemented with 0.3% bovine serum albumin. A virus suspension which contained 4 hemagglutinating units was employed for HI tests. The HI titer of the hyperimmune antiserum (twofold dilution) was expressed as the reciprocal of the highest dilution of antibody which completely inhibited hemagglutination.

Genome RNA extraction and polyacrylamide gel electrophoresis. Genome double-stranded RNA was extracted from the infected MA 104 cell lysates with phenol-chloroform as described previously (9). The RNA was fractionated on a 12% polyacrylamide slab gel with a 3.5% stacking gel, followed by staining with ethidium bromide and photography under UV light as reported previously (9).

RESULTS

Antigenic relationship between a canine rotavirus and the four serotypes of human rotaviruses. Table 1 summarizes the antigenic relationship between canine rotavirus (CU-1) and the four serotypes of human rotaviruses by PRN tests. As previously reported, canine strain CU-1 and two serotypes of human rotavirus (DS-1 and Wa) were serotypically distinct; however, a one-way antigenic relationship in which the CU-1 strain appeared to be the prime strain was recognized between strain CU-1 and the tentatively designated third serotype (M) of human rotavirus (14).

To further study the antigenic relationship between the canine rotavirus (CU-1) and the tentatively designated third human rotavirus serotype, we examined additional strains of the latter (B, no. 14, no. 15, and P, as well as M) by the PRN test (Table 2). The CU-1 strain of canine rotavirus was found to be similar to strains no. 14, no. 15, and P in a reciprocal cross neutralization test, according to the 20-antibody-unit criterion. Strains M and B were related to the CU-1 strain in a one-way fashion, with the latter having the reactivity of a prime strain. No antigenic relationship was found between strain CU-1 and the newly established (15) fourth serotype of human rotavirus (St. Thomas no. 4).

Antigenic relationship between canine and simian rotaviruses by PRN test. To further study the antigenic relationship between canine and simian rotaviruses, three isolates of canine rotavirus (CU-1, A79-10, and LSU 79C-36) and two isolates of simian rotavirus (MMU 18006 and SA11) were compared by the PRN test. There was a close reciprocal antigenic relationship between three strains of canine rotavirus and the two strains of simian rotavirus, indicating that the canine and simian rotaviruses belong to the same serotype (Table 3). There was no serotypic variation among these three strains of canine rotavirus (Table 3).

Comparison of canine and simian rotaviruses by hemagglutination and HI tests. The CU-1 strain of canine rotavirus hemagglutinated hu-

TABLE 1. Antigenic relationship between canine rotavirus and four serotypes of human rotaviruses by PRN

Rotavirus (strain)	Reciprocal of 60% plaque reduction neutralizing titer of hyperimmune antiserum[a] to indicated rotavirus (strain)				
	Canine (CU-1)	Human (Wa)	Human (DS-1)	Human (M)	Human (St. Thomas no. 4)
Canine (CU-1)	**67,350**[c]	<80	<80	295	<80
Human (Wa)	135	**63,365**			
Human (DS-1)[b]	164		**28,365**		
Human (M)[b]	5,357			**30,449**	
Human (St. Thomas no. 4)[b]	<80				**3,895**

[a] Hyperimmune antisera against DS-1 and M were prepared by using rotaviruses grown after genetic reassortment with bovine rotavirus UK strain.

[b] Human bovine reassortant rotavirus.

[c] Homologous values are set in boldface.

TABLE 2. Comparison of canine rotavirus and the tentatively designated third human rotavirus serotype by PRN

Rotavirus (strain)	Reciprocal of 60% plaque reduction neutralizing titer of hyperimmune antiserum[a] to indicated rotavirus (strain)					
	Canine (CU-1)	Human (M)	Human (B)	Human (no. 14)	Human (no. 15)	Human (P)
Canine (CU-1)	**60,443**[b]	313	300	30,477	12,755	27,804
Human (M)[c]	6,042	**29,887**				
Human (B)[c]	4,858		**35,315**			
Human (no. 14)[c]	4,059			**57,302**		
Human (no. 15)[c]	3,737				**70,584**	
Human (P)	5,120					**40,630**

[a] Hyperimmune antisera against M, B, no. 14, and no. 15 were prepared by using rotaviruses grown after genetic reassortment with bovine rotavirus UK strain.
[b] Homologous values are set in boldface.
[c] Human-bovine reassortant rotavirus.

man group O (titer, 1:1,024), rhesus monkey (titer, 1:1,024), dog (titer, 1:1,024), guinea pig (titer 1:128), and sheep (titer, 1:32) erythrocytes. The MMU 18006 strain of rhesus monkey rotavirus hemagglutinated human group O (titer, 1:512), dog (titer, 1:512), and guinea pig (titer, 1:16) erythrocytes, but failed to hemagglutinate rhesus monkey or sheep erythrocytes.

The antigenic relationship between canine and simian rotaviruses was examined by the HI test with human group O erythrocytes (Table 4). The close antigenic relationship demonstrated by the PRN test between canine and simian rotaviruses was confirmed by the HI test (Table 4).

Comparison of canine and rhesus rotavirus strains by gel electrophoresis of viral RNA. To eliminate the possible contamination of one virus with another in the laboratory, migration of segmented viral RNA genes was examined by gel electrophoresis. The CU-1 strain of canine rotavirus had an RNA electropherotype which was distinct from that of the MMU 18006 strain of rhesus monkey rotavirus (Fig. 1). Three isolates of canine rotavirus (CU-1, A79-10, and LSU 79C-36 had similar, but distinct, RNA electropherotypes (Fig. 2).

Subgroup specificity of canine rotaviruses. Strains CU-1, A79-10, and LSU 79C-36 of canine rotavirus were shown to bear subgroup 1 antigen by enzyme-linked immunosorbent assay and immune adherence hemagglutination assay.

DISCUSSION

It has recently been shown that certain human and animal rotaviruses share subgroup (10) or serotype (14) specificity. It has been reported previously (7) that the CU-1 strain of canine rotavirus is similar, if not identical, to the MMU 18006 and SA11 strains of simian rotavirus by the PRN test. This unique antigenic relationship between a canine and simian rotavirus was confirmed by Gaul et al. (3) and again in the present report by using two additional strains (A79-10 and LSU 79C-36) of canine rotavirus that were tested by PRN and HI. As previously reported (7) and confirmed further in this report, the antigenic relatedness between simian (MMU 18006) and canine (CU-1, A79-10, and LSU 79C-36) rotaviruses was closer than that between simian rotavirus (SA11) and the latter. This is in agreement with the recent finding of the antigenic relationship between simian and canine rotaviruses, using monoclonal antibodies directed against the eighth or ninth gene product (primary neutralization determinant) of simian rotavirus MMU 18006 (H. B. Greenberg, J. Valdesuso, K.

TABLE 3. Comparison of canine and simian rotaviruses by PRN

Rotavirus (strain)	Reciprocal of 60% plaque reduction neutralizing titer of hyperimmune antiserum to indicated rotavirus (strain)				
	Canine (CU-1)	Canine (A79-10)	Canine (LSU 79C-36)	Simian (MMU 18006)	Simian (SA11)
Canine (CU-1)	**49,007**[a]	24,574	49,010	65,326	3,754
Canine (A79-10)	34,466	**29,542**	28,109	48,280	3,685
Canine (LSU 79C-36)	28,056	28,517	**31,947**	24,568	4,024
Simian (MMU 18006)	15,654	16,782	20,687	**62,615**	3,255
Simian (SA11)	8,423	9,920	16,658	63,447	**20,536**

[a] Homologous values are set in boldface.

TABLE 4. Comparison of canine and simian rotaviruses by HI test

Antigen (strain)	Reciprocal of HI titer of hyperimmune antiserum to indicated rotavirus (strain)				
	Canine (CU-1)	Canine (A79-10)	Canine (LSU79C-36)	Simian (MMU 18006)	Simian (SA11)
Canine (CU-1)	**5,120**[a]	5,120	5,120	10,240	160
Canine (A79-10)	10,240	**10,240**	2,560	10,240	160
Canine (LSU 79C-36)	10,240	2,560	**2,560**	2,560	80
Simian (MMU 18006)	2,560	2,560	2,560	**40,960**	160
Simian (SA11)	2,560	1,280	2,560	40,960	**1,280**

[a] Homologous values are set in boldface.

van Wyke, K. Midthun, M. Walsh, V. McAuliffe, R. G. Wyatt, A. R. Kalica, J. Flores, and Y. Hoshino, J. Virol, in press). The significance of this observation remains to be elucidated. Rotavirus serotype crossing of species boundaries has also been observed with a simian rotavirus and a tentatively designated third human rotavirus serotype (14); it was also observed with a porcine rotavirus and an equine rotavirus (Y. Hoshino, R. G. Wyatt, H. B. Greenberg, A. R. Kalica, J. Flores, and A. Z. Kapikian, submitted for publication). In the present report, a canine rotavirus was demonstrated to belong to the tentatively designated third human rotavirus serotype, into which simian rotaviruses are grouped. The observations made in this report confirmed and extended the finding that both human and animal rotaviruses can share the same serotype antigen. A systematic serotype classification of both human and animal rotaviruses is under way in this laboratory.

Very recently, Wyatt et al. (15) reported that there are at least four distinct human rotavirus serotypes based on the criterion of a 20-fold or greater difference between homologous and heterologous reciprocal neutralizing titers discriminated by the PRN test. As reported previously (7) and confirmed further in this report, a significant antigenic relationship was not found between the CU-1 strain of canine rotavirus and either Wa (serotype 1) or DS-1 (serotype 2) human rotavirus. Also, the CU-1 strain was found to be antigenically distinct by the PRN test from the St. Thomas no. 4 strain of the

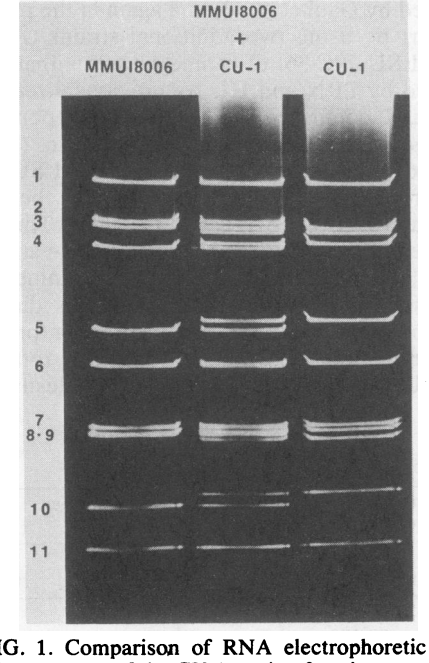

FIG. 1. Comparison of RNA electrophoretic migration patterns of the CU-1 strain of canine rotavirus and the MMU 18006 strain of simian rotavirus. Migration was from top to bottom, and genome segments are numbered in descending order of molecular weight.

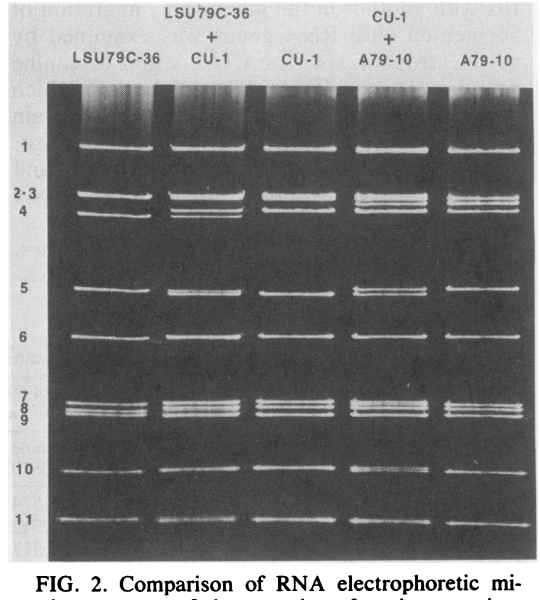

FIG. 2. Comparison of RNA electrophoretic migration patterns of three strains of canine rotavirus (CU-1, LSU 79C-36, and A79-10).

newly defined fourth human rotavirus serotype (15).

The efficient hemagglutinating activity of the CU-1 strain of canine rotavirus makes the HI test an easy and inexpensive laboratory assay for determining the prevalence of antibody to canine rotavirus in the dog population. This may be of epizootic importance, since the HI test detects more type-specific antigens than complement fixation assay, fluorescent-antibody assay, and enzyme-linked immunosorbent assay, which detect group-specific antigens of rotavirus. In addition, this test may be useful in epidemiological studies in which the canine rotavirus is used as a substitute antigen for the third human rotavirus serotype.

Although no serotypic variation was detected among the three isolates of canine rotavirus employed in this study (two from New York and one from Louisiana), more than one serotype of canine rotavirus may exist. Multiple rotavirus serotypes have been reported in humans (11–15), pigs (1), and cattle (S. K. Gaul, N. E. Kelso, F. Simpson, and G. N. Woode, Abstr. Conf. Res. Workers Anim. Dis., 111, p. 21, 1982).

The canine rotaviruses seem to be less pathogenic in terms of diarrheagenic potentiality in the dog population than canine parvovirus (2) and canine coronavirus (2). The canine rotavirus which was found to belong to the third human rotavirus serotype may have potential use as a vaccine not only in dogs but also in humans, if it is attenuated for humans but is capable of inducing effective immunity.

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