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Group C Rotaviruses in Humans

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Atypical rotaviruses obtained from human feces from Australia, Brazil, and the United Kingdom were shown by a combination of techniques—immunoelectron microscopy, immunofluorescence, genome profile analysis, terminal fingerprint analysis of genome segments, and dot-blot hybridization—to be related to group C porcine rotaviruses. The prevalence of antibody to group C rotaviruses was found to be low in human sera and immunoglobulin pools from six countries. No signs of infection were obtained when one of the human viruses was inoculated into gnotobiotic piglets. We conclude that the atypical human viruses are the first examples of group C rotaviruses in humans.

The role of rotaviruses in childhood diarrhea has been established by tests which rely on a common group antigen and by genome profile analysis. Recently, however, viruses with rotavirus morphology but without the common group antigen and usual genome profile have been found in humans (10, 11, 13, 15, 18, 19) and animals (3, 8, 14, 20, 21). These atypical rotaviruses are not recognized by the established diagnostic tests based on serology, and because of the lack of antigenic similarity, it cannot be expected that they will be controlled by the rotavirus vaccines which are currently being developed.

In a study of typical and atypical porcine rotaviruses, Pedley et al. (16) defined three rotavirus groups, A, B, and C, with group A containing the majority of rotaviruses studied to date. Each group possessed its own unique group antigen and genome profile as well as unique terminal fingerprint patterns of the genome segments. To date, all but one of the atypical rotaviruses identified in humans have genome profiles similar to porcine isolates classified as group C, but it is not known whether the atypical human and poricne group C viruses are related either antigenically or by the more rigorous nucleic acid criteria used to define rotavirus groups (9, 16).

The present report examines the antigenic and nucleic acid relationships between two human isolates from Australia and Brazil and group C viruses from pigs. Antigenic relationships were studied by immunoelectron microscopy and immunofluorescence, and nucleic acid relationships were studied by genome profile analysis, dot-blot hybridization, and terminal fingerprint analysis. In addition, antiserum to a third human virus from the United Kingdom was tested by immunoelectron microscopy and immunofluorsecence.

MATERIALS AND METHODS

Viruses. The group A rotaviruses used were the porcine OSU strain (A/OSU) and the bovine UK strain (A/UK) both propagated in cell cultures (4, 16). The group B virus was the porcine NIRD-1 strain, B/NIRD (6).

The group C porcine rotaviruses were the American Cowden and S strains, C/Cowden and C/S (1), and an isolate from the United Kingdom, 37030 (C/37030), identified in association with D. Chasey, Central Veterinary Laboratory, Weybridge, England. The group B and C porcine viruses were propagated in gnotobiotic piglets. The atypical human rotaviruses originated from Australia (strain TG supplied by R. F. Bishop, Royal Children's Hospital, Melbourne) and Brazil (strain 22052 from H. G. Pereira, Instituto Oswaldo Cruz, Rio de Janeiro).

Antisera. Antisera to the porcine viruses were obtained 3 weeks after oral inoculation of gnotobiotic piglets with bacteria-free inocula prepared by either filtration through 0.22- or 0.45-μm membranes (Millipore Corp.) or treatment with (per milliliter) 500 μg of dimetridazole (May and Baker Ltd.) and 1,000 μg each of streptomycin sulfate, benzylpenicillin, and spectinomycin dihydrochloride (Abbott Laboratories). Antiserum to the human Australian virus was prepared by intramuscular inoculation of a rabbit with partially purified virus emulsified in Freund incomplete adjuvant. Antisera to the human Brazilian virus, prepared similarly in rabbits and guinea pigs but with Freund complete adjuvant, were supplied by H. G. Pereira. A guinea pig antiserum to the human UK virus, 5066 (identified in a adult with diarrhea by D. Cubitt, Central Middlesex Hospital, London), was supplied by G. Beards, Regional Virus Laboratory, Birmingham, England. A human convalescent serum to 5066 was supplied by D. Cubitt. Sera from random children were kindly supplied by I. Chrystie from sera submitted to St. Thomas's Hospital, London, in 1982, and sera from random human adults, taken before 1984, were kindly supplied by A. Bryden, Preston Infirmary, Preston, England. Human immunoglobulin pools were obtained from the Blood Products Laboratory, Elstree, Hertfordshire, England, and from D. Cubitt; these pools were produced in or before 1983.

Immunoelectron microscopy. Virus suspensions were prepared from feces by centrifugation (7), and only preparations which had no virus aggregates were used. Suitable virus suspensions (5-μl volumes) were incubated for 2 h at 37°C with equal volumes of dilutions of test sera. Drops of the reaction mixture were placed on carbon-coated Formvar grids, stained with 2% potassium phosphotungstic acid (pH 6.0), and examined in the electron microscope. The sizes and number of virus aggregates were noted, and the last serum dilution producing aggregates was taken as the titer of the serum. Negative controls for sera to the human viruses were

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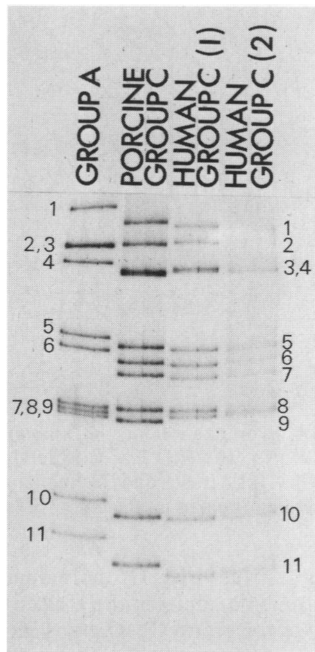

FIG. 1. Genome profiles of porcine A/OSU, porcine C/Cowden, and human Australian [human group C (1)] and Brazilian [human group C (2)] rotaviruses. Numbers on the left and right indicate the positions of the RNA segments of the group A and C viruses, respectively.

preimmune rabbit or guinea pig sera; the negative control for the porcine sera was a convalescent-stage serum to transmissible gastroenteritis virus raised in a gnotobiotic piglet. A positive serum was always included.

Immunofluorescent activity of sera. MA104 cells infected with A/UK or sections of frozen intestinal tissue taken from a gnotobiotic piglet infected with the porcine C/Cowden virus were stained with dilutions of the test antisera followed by the appropriate fluorescein-conjugated anti-species immunoglobulin (Nordic Laboratories Ltd.). Random human sera were tested at 1:20, and immunoglobulin pools were tested at 1:100 and 1:500 dilutions.

Genome profile analysis and terminal fingerprinting. Genomic double-stranded RNA was extracted and labeled at its 3′ termini with ^{32}P-labeled cytidine bis-phosphate and then fractionated on 7.5% polyacrylamide gels run at 20 mA for 16 h by the Laemmli discontinuous buffer system (16). For use in one-dimension terminal fingerprinting, individual RNA segments were fractionated on 40-cm-long 6% polyacrylamide gels (16). Partial digestion of end-labeled RNA segments with RNase T1 and fractionation of the digested fragments on thin 16% gels were carried out as previously described (16).

Dot-blot hybridization analysis. Genomic double-stranded RNA from A/OSU, B/NIRD-1, C/Cowden, C/S, and the human Australian and Brazilian viruses were all dotted onto nitrocellulose, and ^{32}P-end-labeled double-stranded RNA from the human Australian virus was used as the probe. Prehybridization conditions, hybridization, and washing of the filter were all as previously described (17).

Infection of piglets. Gnotobiotic piglets were inoculated orally at 5 days of age with 1.0 to 2.0 ml of bacteria-free inocula prepared from fecal samples. Body weights and appetite were noted and fecal samples were collected before and after inoculation.

RESULTS

Genome profile analysis. Comparison of the Australian and Brazilian human viruses with the porcine A/OSU and C/Cowden viruses showed that the overall pattern of migration of the genome segments of the human viruses was very similar to that of the porcine group C virus (Fig. 1). Although some minor differences in the migration of some of the segments were evident, both human viruses had a group of three genome segments migrating in the region of segments 5 and 6 of group A rotaviruses and a pair of segments migrating in the region of segments 7, 8, and 9 of group A rotaviruses. In addition, the porcine C/S and C/37030 viruses and the human UK virus exhibited this characteristic pattern (16; D. Chasey and D. Cubitt, personal communication).

Antigenic relationships. By immunoelectron microscopy, the human and porcine viruses were found to be related (Table 1). Antisera to the two porcine viruses, C/Cowden and C/37030, cross-reacted with the human Australian virus or the Australian and Brazilian viruses. In the reciprocal cross, antisera to the human Australian and UK viruses cross-reacted with the porcine C/Cowden virus. However, two antisera to the human Brazilian virus failed to react with the C/Cow-den virus. These sera were supplied as unreactive in a homologous enzyme-linked immunosorbent assay, although, surprisingly, in the present study they were reactive by immunoelectron microscopy with both the Brazilian and Australian human viruses. With all three antigens, both double- and single-shelled virions were agglutinated, but it was impossible to say whether agglutination of double-shelled particles was due to cross-reactive antigens on the outer capsid layer or whether breaks in the outer layer had exposed inner antigens which were masked in intact double-shelled particles (2).

The cross-reactions with the porcine antigen were also examined by immunofluorescence; sera to the Australian and UK human viruses cross-reacted to at least 1:20 dilutions, whereas there was an equivocal reaction at 1:20 dilution with the guinea pig serum to the Brazilian virus.

Terminal fingerprinting and dot-blot hybridization studies. Comparison of the terminal fingerprint patterns of C/Cowden and the Australian human virus for genome segments 6 and 7 indicated a high level of conservation in the region 8 to 40 nucleotides from the termini, with the majority of differences

TABLE 1. Immunoelectron microscopy with atypical human and group C porcine rotaviruses

Antiserum to:	Titer of virus		
	Porcine (C/Cowden)	Human	
		Brazilian	Australian
Control[a]	<20	<20	<20
Porcine			
C/Cowden	2,560[b]	>80[c]	>640
C/37030	>20[c,d]	NT	>80
Human			
Brazilian	<10[c]	>80[c]	80[c]
Australian	>160	NT	>640
UK	40	NT	>160

[a] Antiserum to transmissible gastroenteritis virus or preimmune rabbit or guinea pig sera.
[b] The endpoint was read as the last serum dilution which produced virus aggregates. Homologous titers are underlined.
[c] Results with two antisera.
[d] By immunofluorescence.

being only in the intensities of the bands (Fig. 2). Similar fingerprint patterns in this region were also found for the other corresponding genome segments of these two viruses, although with some segments the patterns were not as highly conserved as those of segments 6 and 7. However, these differences were minor compared with the differences observed between rotavirus groups (16). As expected for rotaviruses belonging to group C (16), the fingerprint bands closest to the termini were conserved across all of the genome segments of both viruses, although the present results indicate that our previous estimate of 10 nucleotides for its length should be revised to include only the first 8 nucleotides. Unfortunately, there was insufficient double-stranded RNA available for the human Brazilian virus to be analyzed by terminal fingerprinting.

By dot-blot hybridization with a 70% stringency level, the human Australian virus showed a high level of sequence homology with the two porcine group C strains and the human Brazilian virus (Fig. 3). In contrast, there was only a slight cross-reaction with the group A and B RNAs.

Antibody prevalence. Antibody to group C rotaviruses was found to be uncommon in human sera when tested by immunofluorescence with the porcine virus as antigen (Table 2). Of 12 immunoglobulin pools tested from the United Kingdom, the rest of Europe, North America, and Japan, none was positive to group C rotavirus, whereas all were positive to group A rotavirus. Of individual serum samples taken from children and adults in the United Kingdom, 0 and 11% were positive for group C, whereas 93% or more were positive for group A rotavirus. Furthermore, in contrast to the serum to the human UK virus taken from a convalescent adult, the reactions of the random sera were weak at 1:20 dilution. The convalescent-stage serum was strongly positive by immunofluorescence at this dilution and was positive up to 1:160 by immunoelectron microscopy with the porcine virus. Unfortunately, preimmune serum was not available to confirm seroconversion.

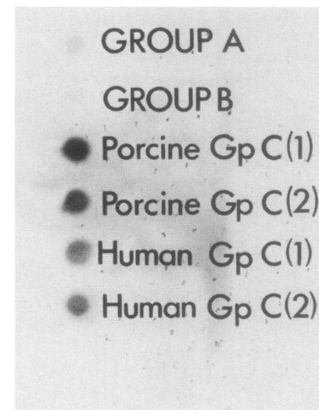

FIG. 3. Dot-blot hybridization of the human Australian virus with the porcine A/OSU, B/NIRD-1, C/Cowden [porcine Gp C (1)], and C/S [porcine Gp C (2)] viruses and the human Australian [human Gp C (1)] and Brazilian [human Gp C (2)] viruses.

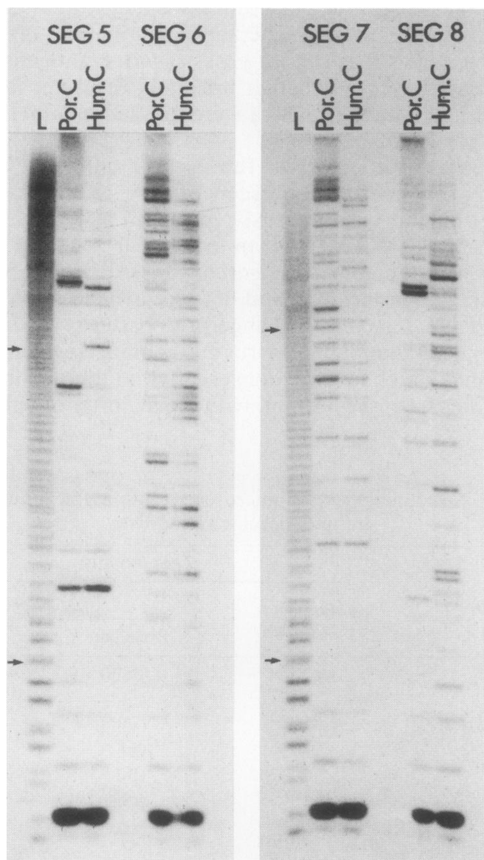

FIG. 2. Terminal fingerprint patterns of genome segments 5, 6, 7, and 8 for C/Cowden (Por.C) and the human Australian (Hum.C) rotaviruses. Lane L represents a reference ladder produced by partial alkaline hydrolysis. Arrows indicate nucleotide positions 10 to 40.

Infection of gnotobiotic piglets. Two unsuccessful attempts were made to infect piglets, aged 5 days, with the human Brazilian virus. No clinical signs were observed, and convalescent-stage sera taken 3 weeks after inoculation failed to react in immunofluorescence tests with the C/Cowden antigen. Upon subsequent inoculation of one 3-week-old piglet with the porcine C/Cowden virus, diarrhea resulted, and group C antibody was detected in convalescent-stage sera.

DISCUSSION

Three atypical rotaviruses from humans were shown to be related to group C rotaviruses from pigs by antigenic or

TABLE 2. Prevalence in human sera of antibody to group A and C rotaviruses, tested by immunofluorescence with A/UK and C/Cowden as antigens

Source of serum	Date and geographical source	No. tested	No. (%) positive to rotavirus group	
			A	C
Convalescent stage	1983; United Kingdom	1	NT	1
Random children	1982; United Kingdom	15	14 (93)	0 (0)
Random adults	pre-1984; United Kingdom	38	36 (95)	4 (11)
Immunoglobulin pools	pre-1984;			
	United Kingdom	6	6	0
	Belgium	1	1	0
	Switzerland	1	1	0
	United States	1	1	0
	Canada	2	2	0
	Japan	1	1	0

nucleic acid analyses or both. The viruses possessed a common group antigen, had similar genome profiles and terminal fingerprint patterns, and showed a high conservation of genome sequences. Thus, we have shown for the first time that rotaviruses belonging to groups other than group A can infect more than one animal species. Such viruses are not detected by the serological techniques currently being widely used for rotavirus diagnosis, but the present study has shown that they would be detected by diagnostic tests based on the porcine group C viruses.

The group C rotaviruses from humans and pigs may not, however, be identical, as some differences between them were found by both immunoelectron microscopy and terminal fingerprinting. In addition, although the viability of the inoculum to humans was not tested, the human virus failed to infect pigs, as did two other human isolates with genome profiles similar to those of the viruses used in this study (21).

The present serological studies with human sera plus the small survey of Espejo et al. (11) suggest that infection of humans with group C viruses is a rare event, and this finding is supported by the fact that, despite extensive genome profile analysis of human fecal samples, rotaviruses with group C-like profiles have been found only rarely. The low level of antibody to group C rotaviruses in humans contrasts, however, with that found in pigs, as 77% of porcine sera possessed antibody to group C rotaviruses (5). It remains to be established whether antibody to group C rotaviruses is equally uncommon in humans in Australia and Brazil and other countries where rotaviruses with C-type profiles have been identified, whether group C infection in humans is an emerging infection, and whether it is zoonotic. The recent emergence in China of an extremely pathogenic strain of human rotavirus (12, 13) which does not belong to any of the established rotavirus groups serves to emphasize the need for continued vigilance.

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