Location of Type-Specific Antigens in Calf Rotaviruses

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Rotavirus antisera with and without neutralizing activity to calf rotavirus were compared for their ability to agglutinate calf rotavirus particles with and without an outer capsid layer. Particles without the outer capsid layer were agglutinated by antisera with immunofluorescent antibody activity and by antisera with immunofluorescent antibody and neutralizing activity. Particles with the outer capsid layer were agglutinated only by antisera with neutralizing activity. The neutralization test appears to be type specific, and it was concluded that type-specific antigens are associated with the outer capsid layer of rotaviruses. Since particles with the outer capsid layer were not agglutinated by antisera lacking neutralizing activity but possessing immunofluorescent antibody activity, it was concluded that in intact particles the group-specific antigen is masked by the outer capsid layer.

Rotaviruses, which have been isolated from many animal species, share a common antigen demonstrable by immune electron microscopy, gel diffusion, complement fixation, and immunofluorescence tests (8). This common antigen appears to be associated with the inner capsid layer (16). Evidence for serological differences within the rotavirus group has been provided by neutralization tests, since some sera with immunofluorescent antibody (IFA) activity do not possess neutralizing activity (7, 16). The study of Thouless et al. (12) showed that the neutralization test could differentiate between rotaviruses from different animal species. Recently, hemagglutination inhibition and complement fixation tests have also been used to differentiate some rotavirus isolates (6, 17). Thus it appears that there are serological differences between rotaviruses that are detectable by *in vitro* tests.

Two morphological types of rotavirus particles have been observed in preparations from all the animal species in which they have been found. The larger, 65-nm particle appears to be composed of the smaller, 55-nm particle with an additional outer capsid layer (2). For the purpose of this report, the 65-nm particles have been called "complete" particles and the 55-nm particles "incomplete." Several investigators have reported that, in immune electron microscopy tests, complete particles with the outer layer may not be agglutinated by antisera which agglutinate incomplete particles without the outer capsid layer (3, 7, 11, 16, 17). This paper describes investigations on the ability of rotavirus antisera, with or without neutralizing activity to calf rotavirus, to agglutinate calf rotavirus par-

ticles with or without an outer capsid layer, in an attempt to establish whether neutralizing activity, and therefore type specificity in rotaviruses, is associated with the outer capsid layer.

MATERIALS AND METHODS

Virus. The British isolate of calf rotavirus (14) was prepared either from feces of experimentally infected gnotobiotic calves or from infected tissue culture fluids (2). Virus suspensions in which most of the particles either had or did not have an outer capsid layer were used in the immune electron microscopy tests. Suspensions that consisted mainly of one particle type were used without further centrifugation, but when both complete and incomplete particles were present they were separated in a cesium chloride density gradient (2). Virus suspensions were used within a week of preparation, and those that had clumps larger than three particles were rejected as unsuitable for the experiment.

Antisera. Antisera were prepared in gnotobiotic piglets to four rotaviruses (two human, one porcine, and one bovine strain) and to transmissible gastroenteritis virus (TGEV). Two human fecal samples, A and B, were pooled and used as one human virus inoculum (human rotavirus AB), and two additional human fecal samples, C and D, were used as another human virus inoculum (human rotavirus CD) (3). Porcine virus SW1/2 (15), a British calf rotavirus (9), and the FS772/70 strain of TGEV, obtained from S. Cartwright, Central Veterinary Laboratory, Weybridge, England, were also used. Piglets were infected orally with a fecal filtrate (0.45- μ m membrane filter) and bled 3 to 4 weeks later. Complete and incomplete rotavirus particles were excreted in the feces of all infected piglets for several days, as determined by electron microscopy. Antisera were heated at 56°C for 30 min and filtered through a 0.45- μ m membrane filter before they were diluted in phosphate-buffered saline

(pH 7.2) for use in immune electron microscopy tests.

Serology. IFA activity in sera was determined using primary calf kidney cover-slip cultures infected with cell culture-adapted calf rotavirus (1). The cultures were stained with varying dilutions of the antiserum to be tested, followed by fluorescein-conjugated rabbit anti-swine immunoglobulin (Nordic Laboratories Ltd.).

Neutralizing activity of sera was determined against 100 to 1,000 50% tissue culture doses of the British isolate of calf rotavirus adapted to replicate in cell cultures (1, 14).

Immune electron microscopy. In experiment 1, antiserum to human rotavirus AB was compared with antiserum to human virus CD in its ability to agglutinate complete and incomplete rotavirus particles. In experiment 2, antiserum to calf rotavirus was compared with pig rotavirus antiserum. Antiserum to TGEV was used as a control serum. Volumes of 10 μ l of a suspension of calf rotavirus particles were added to equal volumes of 1/10, 1/20, and 1/40 dilutions of antisera. The mixtures were incubated for 1 h at 37°C and then overnight at 4°C before drops were placed on carbon-coated Formvar grids, stained with 2% phosphotungstic acid (pH 6.0), and examined in a Philips 300 electron microscope. Between 250 and 1,000 virus particles were counted for each virus-serum mixture, and the number and size of aggregates were noted. Clumps of more than three particles were taken to indicate that agglutination had occurred.

RESULTS

IFA and neutralizing activity of sera. Antisera to human rotaviruses AB and CD used in experiment 1, and the antisera to calf and pig rotaviruses used in experiment 2, had IFA titers of between 160 and 320 (Table 1). Only two of these antisera, that to human rotavirus AB and that to calf rotavirus, possessed neutralizing activity to calf rotavirus. The control TGEV antiserum was devoid of IFA and neutralizing activity to calf rotavirus.

Agglutination of incomplete calf rotavirus particles. In experiments 1 and 2, the four antisera with IFA to calf rotavirus agglutinated

incomplete particles (Table 2). At the serum dilutions used, the majority of particles were aggregated into clumps larger than three particles, whereas no particles were seen to be clumped in the control TGEV antiserum mixture.

Agglutination of complete calf rotavirus particles. In experiment 1, where antisera to human rotaviruses were compared, only antiserum to human rotavirus AB (which had neutralizing activity) agglutinated complete calf rotavirus particles (Table 3). Antiserum to human virus CD, which was devoid of neutralizing activity to calf rotavirus at a 1/10 dilution, reacted differently; only one aggregate was observed. Similarly, in experiment 2, antiserum to pig rotavirus, which did not have neutralizing activity, failed to agglutinate complete particles, whereas calf rotavirus antiserum agglutinated these particles. Rotavirus particles were not agglutinated by antiserum to TGEV. Results similar to those shown for experiment 1 were obtained on three separate occasions, and results similar to those shown for experiment 2 were obtained on two occasions.

TABLE 1. IFA and neutralizing activity of antisera to calf rotavirus

Expt	Antiserum to:	Titers to calf rotavirus	
		IFA	NT ^a
1	Human rotavirus AB	160	160
	Human rotavirus CD	160	<10
2	Calf rotavirus	320	80
	Pig rotavirus	320	<10
Control	TGEV	<10	<10

^a NT, Neutralizing activity.

TABLE 2. Agglutination of incomplete calf rotavirus particles by antisera with and without neutralizing activity to calf rotavirus

Expt	Antiserum to	No. of aggregates ^a containing (no. of particles):				% Virus particles in aggregates of:	
		1-3	4-10	11-21	>21	≤3	>3 ^b
1	Human rotavirus AB	78	20	5	0	31	69
	Human rotavirus CD	59	24	11	4	18	82
2	Calf rotavirus	54	48	5	1	16	84
	Pig rotavirus	94	57	12	3	18	82
Control	TGEV	259	0	0	0	100	0

^a The sum of the numbers of aggregates seen in the serum dilutions used.

^b The presence of aggregates of more than three virus particles was taken to indicate that agglutination had occurred.

TABLE 3. *Agglutination of complete calf rotavirus particles by antisera with and without neutralizing activity to calf rotavirus*

Expt	Antiserum to:	NT titer ^a	No. of aggregates ^b containing (no. of particles):				% Virus particles in aggregates of:	
			1-3	4-10	11-21	>21	≤3	>3 ^c
1	Human rotavirus AB	160	53	22	12	3	16	84
	Human rotavirus CD	<10	249	1	0	0	97	3
2	Calf rotavirus	80	339	30	4	1	52	48
	Pig rotavirus	<10	395	0	0	0	100	0
Control	TGEV	<10	351	0	0	0	100	0

^a NT, Neutralizing activity.^b The sum of the numbers of aggregates seen in the serum dilutions used.^c The presence of aggregates of more than three virus particles was taken to indicate that agglutination had occurred.

DISCUSSION

Complete calf rotavirus particles with an outer capsid layer were agglutinated by rotavirus antisera with neutralizing activity but not by antisera without neutralizing activity to calf rotavirus. Thus the antigen responsible for stimulating neutralizing activity is associated with the outer capsid layer of rotaviruses. This result correlates with the observation that it is the complete particles that are associated with the infectivity of rotaviruses (2, 4).

The results with sera with IFA activity but without neutralizing activity confirm that the former activity, by which all rotaviruses are related, is associated with incomplete particles. Because these sera failed to agglutinate complete particles, this group-specific antigen must be masked in intact, complete particles. However, since most preparations of rotaviruses contain particles with, without, or with only a part of an outer layer, group- and type-specific reactions can be expected.

The potential problem that partial degeneration of the outer capsid layer of the complete particles would reveal inner antigens and make the test impracticable was not encountered. This was probably because freshly prepared virus preparations were used in the immune electron microscopy tests. A break in the outer capsid layer may account for the single clump of particles observed in experiment 1 with antiserum to human rotavirus CD.

Neutralization tests can differentiate between rotaviruses from different animal species (12), but some observations have indicated that more than one type is present in some species. The antisera to the two human rotavirus isolates used in this study had different serological characteristics and, since only a proportion of random sera from human or porcine populations

with IFA activity had neutralizing activity to calf rotavirus, it appears that there are serological differences between rotaviruses within these populations (16). The presence of different types of rotavirus in the human population has been confirmed by serology (12, 17) and by RNA analysis (5, 10). Thus it seems that it is a type-specific antigen, rather than a species-specific antigen, that is detected by neutralization and that is determined by the outer capsid layer of the virus.

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