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Analysis of the Structural Polypeptides of a Porcine Group C Rotavirus

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Polyacrylamide gel analysis of the structural polypeptides of purified group C virions allowed six major proteins to be identified. Of these, two (52,000- and 39,000-molecular-weight polypeptides) were shown to be in the outer virion shell as judged by the ability to strip them from virions by treatment with EDTA. Treatment of purified particles with endo-β-*N*-acetylglucosaminidase F showed that the 39,000-molecular-weight outer shell polypeptide is probably posttranslationally glycosylated. Serological cross-comparison of groups A and C by using Western blotting (immunoblotting) extended the previously demonstrated lack of cross-reaction for the group antigen to show that none of the structural polypeptides cross-reacted. Possible implications of these findings for the epidemiology of rotaviruses are discussed.

Rotaviruses are the predominant etiological agents of acute viral gastroenteritis in the young of many animal species, including humans, and as such are major medical and veterinary pathogens (5, 7). Until recently, all rotavirus isolates, irrespective of the host species from which they were isolated, shared a common group antigen, the detection of which forms the basis of all the commercial diagnostic kits that are currently available (20). Recently, however, a number of viruses have been isolated from outbreaks of viral gastroenteritis in various species, including humans, which, despite possessing the characteristic rotavirus morphology, failed to react in assays detecting the group antigen (1, 4, 6, 8, 13, 17, 18). In addition to this serological distinction, these atypical viruses had characteristic differences in their genome segment distributions on polyacrylamide gels. By using these differences coupled to changes in their terminal fingerprint patterns, these atypical viruses have been classified into four new rotavirus groups (B to E) (16). In the present report, we compare the structural polypeptides of a porcine group C virus purified from infected feces with those of a typical (group A) rotavirus.

The group C virus used in this study was grown in gnotobiotic piglets since until very recently this virus had not been adapted to routine growth in tissue culture (19). Twenty grams of infected feces was diluted in 100 ml of 50 mM PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)] buffer (pH 6.6) and extracted several times with fluorocarbon (Freon 113; Du Pont). The clarified supernatant from these extractions was concentrated by centrifugation (Beckman SW27 rotor, 1 h, 25,000 rpm). The virus-containing pellet was suspended in 3 ml, and cesium chloride was added to a final buoyant density of 1.37. Virus was banded by isopycnic centrifugation (Beckman TL100 centrifuge, 3 h, 100,000 rpm), and the single visible virus band was collected, diluted in PIPES buffer, and stored at −20°C until used. The group A virus (bovine RF strain) (11) used in this study was purified from infected MA104 cells as previously described (3).

The purity of the virus samples was assessed by electron microscopy after negative staining with uranyl acetate, and, in agreement with others, the group A and group C viruses proved to be morphologically indistinguishable (data not shown). The virions examined were predominantly double-shelled particles.

Comparative analysis of the structural proteins of the group A and C viruses was carried out on a 12.5% polyacrylamide sodium dodecyl sulfate-polyacrylamide gel by using the Laemmli discontinuous buffer system (10). Figure 1 shows this gel after staining with silver nitrate (14). Five major protein bands ranging in molecular weight from 125,000 to 37,000 were resolved for each virus. The patterns of the two groups were very similar, the main difference being that the group C protein that corresponds to the major outer shell glycoprotein (VP7) of group A migrated at 39,000 molecular weight as opposed to 37,000 molecular weight for VP7. The outermost of the two concentric capsid shells of group A rotavirus can be removed easily by treatment with EDTA (3), allowing localization of certain virion structural proteins to that shell. To determine whether a similar protein localization was possible with group C virus, 40 μg of purified virus was incubated for 10 min at 20°C in 50 mM Tris hydrochloride buffer (pH 7.5) in the presence or absence of 2 mM EDTA. Following treatment, virus samples were centrifuged at 100,000 rpm for 10 min in an Airfuge (Beckman), and the proteins remaining in the pelleted virions were analyzed by polyacrylamide gel electrophoresis. Figure 2 shows that two group C polypeptides of 39,000 and 52,000 molecular weight were removed by treatment with EDTA, allowing their tentative localization to the outer shell of the virus. Group A rotaviruses have one of their outer shell polypeptides, VP7, which carries some of the neutralization determinants of the virus, posttranslationally modified by glycosylation (9, 12). It was therefore of interest to investigate whether either of the two outer shell proteins of group C was glycosylated. Five micrograms of purified group C virus was incubated at 37°C for 16 h with 0.5 U of endo-β-*N*-acetylglucosaminidase F (Boehringer-Mannheim) in 100 mM sodium acetate (pH 5.0)–50 mM EDTA–1% sodium dodecyl sulfate–150 mM 2-mercaptoethanol. Following this treatment, viral polypeptides were analyzed by polyacrylamide gel electrophoresis, which showed (Fig. 3) that the 39,000-molecular-weight polypeptide suffered an approximately 2,000-molecular-weight decrease, indicating that it is a glycoprotein.

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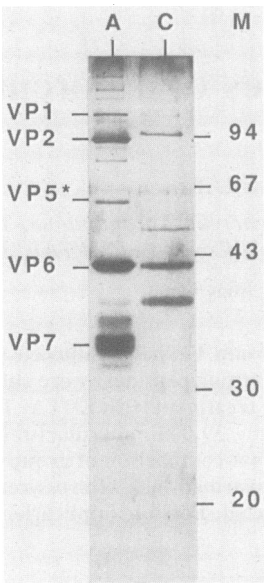

FIG. 1. Comparison of structural polypeptides of group A and C viruses. Five-microgram samples of virions purified on cesium chloride gradients were analyzed on a 12.5% polyacrylamide gel run by using the Laemmli discontinuous buffer system (10). This gel was electrophoresed at 60 V for 16 h and then stained with silver nitrate (14). Lanes: A, double-shelled group A virions; C, double-shelled group C virions. The designations on the left side of the figure refer to some of the major structural proteins of group A virions. Lane M, molecular weight markers (in thousands).

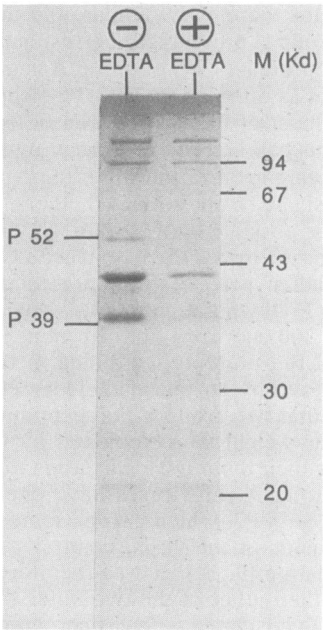

FIG. 2. Localization of group C virion polypeptides to the outer capsid shell. Two 40-μg samples of purified group C virions were incubated for 10 min at 20°C in 50 mM Tris hydrochloride buffer (pH 7.5) with or without 2 mM EDTA and then pelleted by centrifugation for 10 min in a Beckman Airfuge (100,000 rpm). Polypeptides in the viral pellets were then analyzed on a 12.5% polyacrylamide sodium dodecyl sulfate-polyacrylamide gel. After electrophoresis as described in the legend to Fig. 2, the gel was stained with Coomassie brilliant blue R250. EDTA-treated virus is shown on the right, and untreated virus is shown on the left.

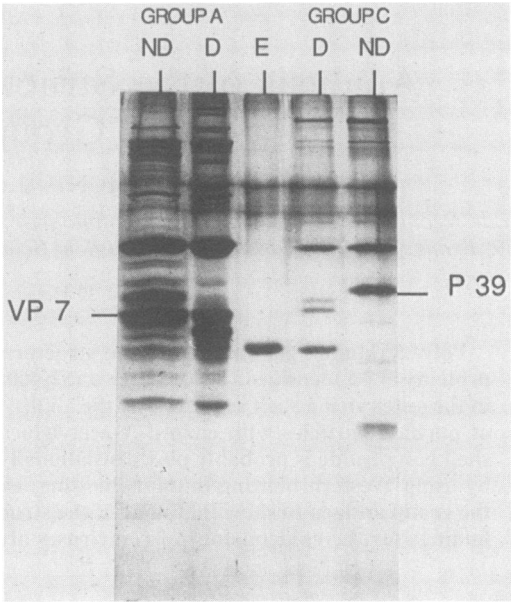

FIG. 3. Glycosylation of the 39,000-molecular-weight outer shell polypeptide. Five micrograms of purified group A or C virions was digested for 16 h at 37°C with 0.5 U of endo-*N*-acetylglucosaminidase F before being analyzed on a 12.5% polyacrylamide sodium dodecyl sulfate-polyacrylamide gel as described in the legend to Fig. 2. Lanes: ND, not digested; D, digested. A sample of the endo-β-*N*-acetylglucosaminidase F used for the digestion was run in lane E as a control. The migration positions of VP7 of group A and of the 39,000-molecular-weight outer capsid shell protein of group C are indicated.

Serological cross-comparison of group A and C viruses has been performed by indirect immunofluorescence (15, 16) using polyclonal antibodies. This test, which detects essentially the group antigen, VP6, and, to a lesser extent, other viral antigens, has shown that group A and C viruses do not cross-react. It was of interest to confirm this finding by using a Western blot (immunoblot) analysis with hyperimmune antisera against the two viruses. The results (Fig. 4) showed that the group A sera failed to react with any of the proteins present in purified group C virions and that the converse was also true. This confirmed that the lack of cross-reaction between these two viruses is not confined solely to the group antigen (VP6) but extends to all the virion structural proteins.

This study is the first to describe the structural polypeptides of a non-group A rotavirus and has shown that, in line with its morphological similarity to group A viruses, the group C virus examined had a similar complement of structural polypeptides arranged in two concentric shells. The presence of a glycoprotein in the outer shell of the virion is a further indication of similarity to its group A counterpart. Given that this glycoprotein is the major neutralization antigen in group A viruses, the 39,000-molecular-weight glycoprotein identified here will be a focus for further studies on the group C viruses. Despite this overall similarity in polypeptide constitution, the Western blot analysis failed to show any serological cross-reaction between the polypeptides of these two viruses. Previous nucleic acid-based cross-comparisons using terminal fingerprint analysis (15) and dot-blot hybridization with cDNA probes to group A genome segments (2) also showed that there were major differences between these viruses at the nucleic acid level.

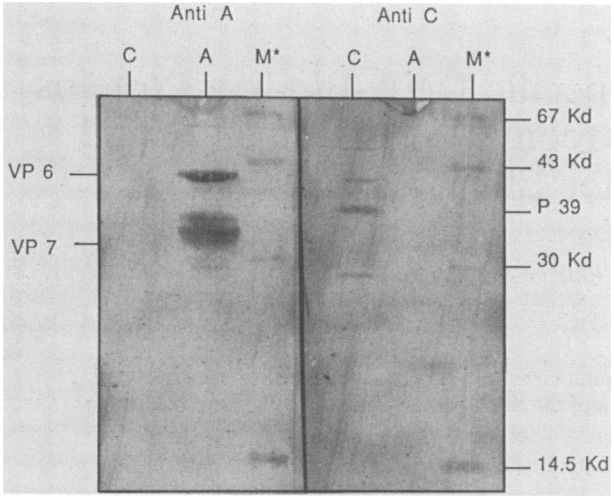

FIG. 4. Western blot analysis. Gels were electrotransferred in duplicate on 2 nitrocellulose membrane in Tris-glycine buffer containing 20% methanol for 3 h at 250 mA. Lanes: C, group C rotavirus; A, group A rotavirus; M, ^{14}C-labeled molecular weight markers. Blots were incubated for 1 h at room temperature with antisera diluted (1/100 for anti-A, 1/20 for anti-C) in 50 mM Tris hydrochloride (pH 8)–150 mM NaCl–3% bovine serum albumin. They were then incubated with ^{35}S-labeled protein A for 1 h at room temperature. Blots were autoradiographed overnight.

Taken together, these data suggest that, despite some similarities between group A and C rotaviruses, they are unlikely to genetically interact with each other in nature. Therefore, their individual contributions to the overall problem of acute infantile gastroenteritis should be assessed independently.

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