Polypeptide Composition of Rotavirus Empty Capsids and Their Possible Use as a Subunit Vaccine

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Two types of empty capsid particles that differed with respect to the presence of the two outer shell proteins were isolated from MA-104 cells infected with bovine rotavirus V1005. Three previously uncharacterized polypeptides, I, II, and III, migrating between VP2 and VP6, were detected in empty capsids but not in single- and double-shelled rotavirus particles. Peptide mapping revealed that all three proteins were related to VP2. Polypeptides I, II, and III could be generated by in vitro trypsin digestion of empty capsids not exposed to trypsin in the infection medium. Labeled polypeptides appeared in empty capsids before they were detected in intracellular single- or double-shelled rotavirus particles. Empty capsids were also observed in MA-104 cells infected with bovine rotaviruses UK and NCDV, simian rotavirus SA11, and human rotavirus KU. VP7-containing empty capsid is the minimal subunit vaccine for cows; we failed to induce a substantial neutralizing antibody increase with VP7 purified under denaturing or non-denaturing conditions or with synthetic peptides corresponding to two regions of VP7.

Rotaviruses are a major cause of diarrhea in young animals and children (19). Therefore, efforts are being made to develop an efficient and safe rotavirus vaccine for infants. Current strategies for the development of rotavirus vaccines are based on the Jennerian approach, which takes advantage of the antigenic relatedness of human and animal rotaviruses and the diminished virulence of live attenuated animal rotavirus strains for humans (21). In accordance with this concept, two bovine rotavirus strains (12, 40) and one rhesus monkey rotavirus strain (15) were tested in several studies in humans. Recently, human-rhesus monkey rotavirus reassortants have also been studied in infants (16). On the other hand, several noninfectious rotavirus vaccines have been investigated. Small laboratory animals have been successfully immunized with isolated rotavirus polypeptides purified from whole rotaviruses (5, 22, 28, 33), with rotavirus polypeptides expressed in bacteria (2, 3, 29), with baculovirus-expressed rotavirus polypeptide (27), with vaccinia virus recombinants (1), and with synthetic peptides (38). Neutralizing antibody titers were, with one exception (27), low, probably because of altered structural integrity of the viral antigens and not their noninfectious nature, because replication of rotavirus in intestinal epithelial cells is not required to induce rotavirus-neutralizing antibodies. Female adult mice parenterally inoculated with chemically inactivated rotavirus (31) or noninfectious purified empty capsids (35) developed neutralizing antibodies, and newborn mice from these dams were protected against rotavirus challenge.

Chemical inactivation altered the structural integrity of the viral outer capsid (31). Empty capsids, on the other hand, are noninfectious because of natural lack of viral RNA despite the presence of rotavirus antigens in a native conformation. Biochemical characterization of rotavirus empty capsids is, however, scarce (9, 11, 35). It is only known that empty capsids contain, in addition to putative VP2, VP5, VP6, and VP7, polypeptides not detected in single-shelled (SS) and double-shelled (DS) particles (9, 11, 35). In this report, we give a biochemical description of extracellular and intracellular empty capsids, characterize the additional polypeptides as VP2 derivatives, and demonstrate that empty capsid is the minimal subunit vaccine for milking cows.

MATERIALS AND METHODS

Viruses. Bovine rotaviruses V1005 (4, 9), NCDV, and UK were obtained from P. A. Bachmann, University of Munich, Munich, Federal Republic of Germany, simian rotavirus SA11 was obtained from P. A. Offit, Children's Hospital of Philadelphia, Philadelphia, Pa., and human rotavirus KU was obtained from S. Urasawa, Sapporo Medical College, Sapporo, Japan.

Cell culture. MA-104 (rhesus monkey kidney) cells were grown in Eagle minimal essential medium containing 10% fetal calf serum. Confluent cell monolayers were washed three times with phosphate-buffered saline (PBS; pH 7.2) and inoculated with bovine rotavirus V1005 at a multiplicity of infection of 20 50% tissue culture infectious doses per cell. The rotavirus was incubated with porcine trypsin (20 μg/ml; Flow Laboratories) for 30 min at 37°C before infection. After 1 h of adsorption at 37°C, cells were washed twice with PBS and incubated in minimal essential medium containing 10% tryptose phosphate (Flow) and 10 μg of trypsin per ml (infection medium).

Extracellular rotavirus. Cells were labeled throughout the entire infection cycle with [35S]methionine (15 μCi/ml; Dupont, NEN Research Products) in infection medium containing 1/10 the normal methionine content.

After destruction of the cell monolayer, rotavirus particles were recovered from the cell-free culture supernatant by high-speed centrifugation (90,000 × g, 2 h, 4°C) through a 20% sucrose cushion. The crude virus pellet was then purified by CsCl equilibrium centrifugation on preformed CsCl gradients (1.25 to 1.42 g/ml). Rotavirus particles banding at 1.31 g/ml (55-nm empty capsids [EC1]), 1.315 g/ml (65-nm empty capsids [EC2]), 1.36 g/ml (DS particles), and 1.38 g/ml (SS particles) were recovered from the CsCl gradients with a pasteur pipette, diluted with TNC (50 mM Tris hydrochloride [pH 7.5] containing 150 mM NaCl and 10 mM CaCl2) and pelleted by a further high-speed centrifugation step (130,000 × g, 1 h, 4°C). Rate zonal sedimentation...
was done on a preformed sucrose gradient. A five-step gradient ranging between 10 and 30% sucrose in PBS was prepared and stored overnight at 4°C. The empty capsid fraction recovered from CsCl gradients and pelleted by a high-speed centrifugation step was loaded on the gradient and centrifuged for 40 min at 30,000 rpm (150,000 × g) in an SW40 rotor (International). The gradient was fractionated into 26 450-μl fractions, and 50 μl was counted by liquid scintillation counting. Peak fractions were pooled, concentrated by a further high-speed centrifugation step, and analyzed by polyacrylamide gel electrophoresis (PAGE).

Control experiments. Cells were labeled with [3H]Uridine (15 μCi/ml; Amersham Corp.), and extracellular viral particles were analyzed on CsCl gradients, which were fractionated and measured in a liquid scintillation counter. No [3H]uridine-labeled material above background level was detected at the density position of empty capsids. No viral RNA was detected at this density position by a sensitive silver staining procedure (18), proving the absence of cross-contamination of infectious RNA-containing particles. The empty capsids were not infectious for MA-104 cells in the infectivity titration test of Sato et al. (34).

On electron microscopic examination, the empty capsid preparation was observed to be free of contaminating cellular membranes. In addition, no labeled cellular polypeptides were detected on autoradiography when empty capsids were isolated from cells labeled with [35S]methionine before infection.

Autoradiographs and dried gels stained with Coomassie blue stain were analyzed by scanning. With the exception of VP1 and VP3, the autoradiographic intensity of the different viral polypeptides was proportional to their Coomassie blue staining.

Intracellular rotavirus. Eight hours after infection (exponential growth phase of the virus, maximal incorporation of [35S]methionine into viral polypeptides, cytopathic effects not yet visible), cells were labeled with [35S]methionine (15 μCi/ml, >800 Ci/mmol; Dupont, NEN) in methionine-free minimal essential medium (Seromed). Immediately after the pulse or pulse-chase labeling period indicated in the text, the cells were scraped off, washed in ice-cold PBS, and suspended in TNC containing 0.5% of the nonionic detergent Nonidet P-40. After medium-speed centrifugation (10,000 × g, 15 min, 4°C), a crude virus pellet was prepared by high-speed centrifugation (130,000 × g, 1 h, 4°C), followed by CsCl equilibrium centrifugation on preformed CsCl gradients.

PAGE. Protein electrophoresis was carried out in slab gels by the method of Laemmli (25), using 13% running and 3% stacking gels. Gels were fluorographed with Enlightning (Dupont, NEN), dried, and exposed on X-ray films (YAR-5; Eastman Kodak Co.).

Limited proteolysis. The method of limited proteolysis analysis followed exactly the digestion procedure for proteins in gel slices described by Cleveland et al. (14). A 10-μl amount of Staphylococcus aureus V8 protease (type XVII; Sigma Chemical Co.) at a concentration of 1 μg/μl was added to each slot. V8 protease cleaves at the COOH-terminal side of aspartic and glutamic acid residues (14).

Preparation of VP7 antigens and immunization of cows. One milking cow was immunized with purified DS rotavirus SA11 corresponding to about 100 μg of protein as determined by the method of Bradford (8). VP7 represented about 30% of the total proteins as assayed by Coomassie blue-stained polyacrylamide gel analyzed by reflectance with a Camag II TLC-Scanner coupled to a computing integrator (Spectra Physics SP 4100). Another milking cow was immunized with an empty capsid preparation of rotavirus SA11 (about three times more total protein than for the DS virus but a comparable amount of VP7 by Coomassie blue staining). Nondenatured purified VP7 antigen was obtained from about 100 μg of DS virus SA11 treated with 10 mM EDTA. After 30 min of incubation at room temperature, EDTA-treated and control virions treated with 10 mM CaCl2 were spun down by high-speed centrifugation (130,000 × g, 1 h, 4°C). Pellet and supernatant were separated and mixed with adjuvant. Individual structural polypeptides from crude extracellular rotavirus SA11 (2 mg of protein, as determined by the method of Bradford (8)) were purified by preparative PAGE using 10% running gels. The separated viral polypeptides were localized by Coomassie blue staining of gel strips cut at both lateral sides. The localized bands were excised with a razor blade and ground in a mortar. This suspension was then mixed with adjuvant without further processing. As analyzed by gel scanning, VP7 represented about 10% of total protein. Immunization of the milking cows, enzyme-linked immunosorbent assay (ELISA), and neutralization tests were done as described previously (10).

A 6-mg amount of the synthetic peptide corresponding to the eight carboxy-terminal amino acids of VP7 from rotavirus SA11 and 8 mg of the synthetic peptide corresponding to amino acids 95 to 103 of VP7 from rotavirus Wa were coupled to 10.5 mg of bovine serum albumin as described previously (39). Half of the conjugate was suspended in complete Freund adjuvant (Difco Laboratories) for the first injection, and the other half was suspended in incomplete Freund adjuvant (Difco) for the second injection. The cows were immunized by two subcutaneous injections.

RESULTS

Extracellular EC1. Previously we reported (9) that two types of empty capsids differing in morphology and polypeptide composition could be isolated from the supernatant of MA-104 cells infected with bovine rotavirus V1005. The EC1 particles contained two polypeptides that comigrated with VP2 and VP6 of SS particles (Fig. 1A). The identity of the putative VP2 and VP6 of EC1 with authentic VP2 and VP6 of SS particles was demonstrated by peptide mapping (Fig. 1C). EC1 contained three polypeptides, I, II, and III, which were not detected in SS particles (Fig. 1A). We used a two-step procedure of pelleting and density gradient isopycnic centrifugation to purify empty capsids. Under these conditions, empty capsids band at a buoyant density similar to that of soluble proteins. To prove that polypeptides I, II, and III were on the particle and not simply copurifying with the empty capsids, the 55-nm empty particles were further purified by rate zonal sedimentation. Empty capsids showed a homogeneous sedimentation pattern (Fig. 2B), and polypeptides I, II, and III remained associated with the empty capsids (Fig. 2A). In addition, polypeptides I, II, and III of purified EC1 were not susceptible to in vitro trypsin digestion with 480 μg of trypsin per ml (Fig. 1B), whereas soluble polypeptides were degraded (data not shown).

One-dimensional peptide mapping showed for polypeptide II a peptide map identical to that for VP2 (Fig. 1C). Polypeptide I migrated slower on sodium dodecyl sulfate-PAGE than did VP3 of SS particles (Fig. 1A). Because insufficient amounts of radioactivity were incorporated into VP3, we could not prepare a one-dimensional peptide map of VP3 and thus not formally exclude the possibility that polypeptide I is related to VP3. However, polypeptides I and III showed...
very similar peptide maps to that of VP2 (Fig. 1C; see also Fig. 7C for EC1 of rotavirus NCDV). VP1 was not consistently associated with empty capsids; it was identified in only 20 of 31 independent empty capsid preparations.

Extracellular EC2. A second type of empty capsids (EC2) with larger (65 nm) and less well organized particles could be isolated by isopycnic centrifugation (9). EC2 showed polypeptides VP2, VP5 (the tryptic cleavage product of VP4 [13]), VP6, and VP7 as well as polypeptides I, II, and III (Fig. 3A). The identity of the putative VP2, VP5, VP6, and VP7 of EC2 with authentic polypeptides I, II, and III of DS particles was shown by limited proteolysis analysis (Fig. 3B). As seen for EC1, VP2 and polypeptides I, II, and III of EC2 showed very similar if not identical one-dimensional peptide maps (Fig. 3B).

Kinetics of polypeptide incorporation into intracellular viral particles. Pulse and pulse-chase experiments were done to determine whether polypeptides I, II, and III were also detected in intracellular empty capsids. We were also interested in determining the kinetics with which these polypeptides appeared in empty capsids.

MA-104 cells infected with bovine rotavirus V1005 were pulse-labeled at 8 h postinfection with [35S]methionine for 5, 10, 20, and 30 min. Intracellular viral particles were isolated immediately after the pulse period and separated by CsCl gradient centrifugation into empty capsids (EC1 and EC2 were pooled and are referred to below as EC) and DS and SS particles. The incorporation of labeled polypeptides into the different viral particles was revealed by autoradiography after electrophoretic separation of the viral polypeptides (Fig. 4).

Small amounts of labeled polypeptides were detected in EC and SS particles after 5- and 10-min pulses. Labeled VP6, VP2, and polypeptides I and II were clearly detected in EC after a 20-min pulse, whereas in SS particles only VP6 was predominantly labeled. After 30 min of pulse-labeling, also VP2 of SS particles was clearly labeled and radioactive VP6 was detected in DS particles. After 30 min of labeling, VP2 and polypeptides I and II of intracellular EC showed identical peptide maps (data not shown).

Next, the cells were pulse-labeled for 30 min with [35S]methionine and then chased for 30, 60, and 90 min with excess unlabeled methionine. The chase protocol had no marked effect on the polypeptide composition of empty capsids (small amounts of VP7 became detectable; Fig. 5). Monitoring the counts at the density position of empty capsids directly in the CsCl gradients did not reveal a decrease in radioactivity with increasing chase period (data not shown). On the other hand, incorporation of labeled polypeptides into DS particles increased with increasing chase period (Fig. 5). After a 60-min chase period, labeled VP4 and VP7 were clearly detectable in DS particles. Radioactivity increase in DS particles was not, however, accompanied by a radioactivity decrease in SS particles (Fig. 5).

Intracellular EC did not show polypeptide III. In vitro digestion of intracellular EC with trypsin resulted in the appearance of polypeptide III (Fig. 5).
intracellular DS particles with trypsin, however, resulted in complete disappearance of VP4 and appearance of VP5, in accordance with published data (13). Polypeptide II in intracellular EC resisted trypsin digestion (Fig. 5).

**Empty capsids grown in the presence of trypsin inhibitor.** Next, viral particles were grown in the presence of soybean trypsin inhibitor (10 μg/ml) to see whether polypeptides I and II are primary translation products or cleavage products due to the presence of trypsin in the infection medium. Extracellular empty capsids from these experiments differed from extracellular EC1 grown in trypsin. They showed much less radioactivity at the molecular weight positions of polypeptides I and II and no radioactivity in polypeptide III (Fig. 6; compare lanes c, d, and f with lane b). Trypsin inhibitor had no effect on the polypeptide composition of extracellular SS particles (Fig. 6; compare lanes a and e). SS and empty capsid particles grown in the presence of trypsin inhibitor underwent an in vitro trypsin digestion (10 min, 100 μg/ml). Trypsin digestion had only a small effect on SS particles (Fig. 6; compare lanes j and e), but it induced a decrease in radioactivity detected in VP2 and a parallel increase in radioactivity detected in polypeptides I and II but not in polypeptide III (Fig. 6; compare lanes g to i with lanes c, d, and f).

**Empty capsids of other rotavirus strains.** Empty capsids are not a peculiarity of MA-104 cells infected with bovine rotavirus V1005. We detected EC1 in MA-104 cells infected with bovine rotaviruses UK (data not shown) and NCDV (Fig. 7A), simian rotavirus SA11 (data not shown), and human rotavirus KU (Fig. 7B). All strains showed empty capsids containing polypeptides I, II, and III. In all strains, limited proteolysis analysis showed that polypeptides I, II, and III were related to VP2 (Fig. 7C). The different rotavirus strains differed, however, with respect to production of EC2. MA-104 cells infected with human rotavirus KU produced as much EC2 as DS particles (Fig. 7B), whereas no or only low amounts of EC2 were produced in MA-104 cells infected with bovine rotaviruses UK and NCDV, respectively (Fig. 7D).

**Immunogenicity of subunit rotavirus vaccines in milking cows.** A milking cow was immunized with an SA11 empty capsid preparation that showed by Coomassie blue staining a comparable amount of VP7 antigen as a parallel DS SA11 virion preparation (data not shown). Both antigens induced
in cows an approximately 30-fold increase in serum neutralizing antibody titer (Table 1; compare cows 69 and 72). Denatured VP7 protein of rotavirus SA11 purified from a preparative polyacrylamide gel induced only a threefold increase in serum neutralizing antibody (Table 1, cow 48). The result did not differ when nonreduced VP7 was used for immunization (Table 1, cow 49). These VP7-induced serum antibody increases were comparable to that obtained with gel-purified VP5 and VP6 (Table 1, cows 53 and 57), whereas no increase was seen after immunization with gel-purified VP1 and VP2 (Table 1, cows 59 and 62). Gel-purified VP6 induced antibodies reactive with virions in ELISA; this was not the case for any other gel-purified protein (Table 1).

Immunization of cows with a synthetic peptide corresponding to the carboxy-terminal part of VP7 or to the variable region 5 of VP7 induced only a twofold increase in serum neutralizing antibody (Table 1, cows 58 and 57).

By EDTA treatment, a nondenatured VP7 antigen could be recovered from DS SA11 virions. After high-speed centrifugation, EDTA-treated virions showed a selective loss of VP7; soluble VP7 together with minor amounts of VP6 could be recovered from the high-speed supernatant (Fig. 8). This nondenatured VP7 preparation induced only a fourfold increase of ELISA and neutralizing antibody in one cow (Table 1, cow 73), whereas no increase was seen in a cow immunized with a virion stripped of VP7 (Table 1, cow 74). The empty capsids were thus the only subunit rotavirus SA11 antigen that induced in cows a serum neutralizing antibody response comparable to that of DS SA11 virions.

**DISCUSSION**

Rotavirus empty capsids showed polypeptides that had no counterparts in DS and SS rotavirus virions. This was not a peculiarity of our cell culture system but was also observed in AGMK cells infected with simian rotavirus SA11 (35) and in BSC-1 cells infected with bovine rotavirus UK (11). Peptide mapping revealed that the supplementary polypeptides I, II, and III are derived from VP2. Polypeptide III was absent in intracellular empty capsids but could be produced by in vitro trypsin digestion. Polypeptide III was also not detected in extracellular empty capsids grown in the presence of trypsin inhibitor. Thus, polypeptide III probably arose when intracellular empty capsids were released into cell culture supernatant that contained 10 μg of trypsin per ml. Polypeptides I and II, however, were already detected in intracellular empty capsids after short pulse-labeling periods. This result raises the possibility that they are primary
translation products. Interestingly, the initiator codon proposed by Kumar et al. (24) for VP2 of bovine rotavirus RF is followed by two in-frame AUG codons, which could result in a VP2 shorter by about 2 or 4 kilodaltons. None of the three first AUG codons conforms with the consensus sequence of Kozak (23) for a strong initiation site.

Alternatively, VP2 might be proteolytically attacked shortly after incorporation into empty capsids but not after incorporation into SS particles. VP2 is located in the core of the SS particles (6) and the only virion protein showing RNA-binding activity (7). Therefore, VP2 is most likely to interact with RNA during virus assembly. Lack of RNA in empty capsids might force VP2 in a different conformation, which might expose part of it to proteolytic attack. This interpretation is supported by the observation that polypeptides I and II were only weakly labeled in extracellular empty capsids grown in the presence of trypsin inhibitor, but both polypeptides could be readily generated by in vitro trypsin digestion of these particles.

Instead of interacting with viral RNA, VP2 can directly interact with VP6 to form empty capsids without participation of viral RNA. The existence of empty capsids and the in vitro assembly of empty spherical particles from purified VP6 (32) demonstrate the importance of protein-protein interaction for rotavirus assembly. The efficiency of this protein-protein interaction is also demonstrated by the fact that radioactive viral polypeptides appeared in empty capsids before they were observed in RNA-containing particles.

The maturation of rotavirus particles is a complex process. Several of our observations with a bovine rotavirus are directly comparable to data recently reported for MA-104 cells infected with simian rotavirus SA11 (20). In both studies, the incorporation of labeled polypeptides into SS particles occurred rapidly, and in both viral systems the detection of radiolabeled VP2 preceded the detection of radiolabeled VP3 by about 10 min. In both studies, radiolabeled polypeptides appeared in DS particles with a lag time of 10 to 15 min. The radioactivity in the DS simian and bovine rotavirus particles increased with the chase period. We and Kabccenell et al. (20) did not observe a concomitant

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**TABLE 1. Immunization of cows with different subunit vaccines**

<table>
<thead>
<tr>
<th>Cow</th>
<th>Antigen</th>
<th>Neutralization titer against rotavirus</th>
<th>Titer increase (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-immune</td>
<td>Immune</td>
</tr>
<tr>
<td>69</td>
<td>DS virion (positive control)</td>
<td>800</td>
<td>25,000</td>
</tr>
<tr>
<td>72</td>
<td>Empty capsid</td>
<td>400</td>
<td>12,000</td>
</tr>
<tr>
<td>74</td>
<td>EDTA-treated virion (pellet)</td>
<td>1,200</td>
<td>1,200</td>
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<tr>
<td>73</td>
<td>EDTA-treated virion (supernatant)</td>
<td>500</td>
<td>2,000</td>
</tr>
<tr>
<td>59</td>
<td>Gel-purified VP1</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>62</td>
<td>Gel-purified VP2</td>
<td>1,800</td>
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<tr>
<td>53</td>
<td>Gel-purified VP5</td>
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<td>Gel-purified VP6</td>
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<td>48</td>
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<td>Gel-purified VP7</td>
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<td>3,200</td>
</tr>
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<td>58</td>
<td>Synthetic peptide, VP7 (amino acids 319-326, SA11)</td>
<td>800</td>
<td>1,700</td>
</tr>
<tr>
<td>57</td>
<td>Synthetic peptide, VP7 (amino acids 95-103, Wa)</td>
<td>500</td>
<td>1,000</td>
</tr>
</tbody>
</table>

* Each number is the mean of four determinations.

* — No titer increase.

* Serum neutralizing antibody titers were not higher when tested against rotavirus Wa.

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**FIG. 8. PAGE analysis.** DS rotavirus SA11 treated with 10 mM EDTA (E) or 10 mM CaCl2 (C) underwent high-speed centrifuga-

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http://jvi.asm.org/ on October 28, 2017 by guest
decrease in the radioactivity of the SS particles with increasing chase period. This latter observation is hard to reconcile with a simple precursor-product relationship between SS and DS rotavirus particles. For technical reasons, this observation should be interpreted with caution. It has been demonstrated that intracellular rotavirus SA11 was associated with membranes (37) and cytoskeleton (30). Intracellular SS SA11 rotavirus could not be recovered from MA-104 cells extracted with 1% Triton X-100 (30). Confirming these results, we could isolate intracellular DS but not SS rotavirus SA11 from MA-104 cells extracted with 0.5% Nonidet P-40 (H. Brüssow, unpublished results). In contrast, SS and DS particles could be isolated from bovine rotavirus V100S-infected MA-104 cells extracted with 0.5% Nonidet P-40. Our data exclude a precursor role for the SS particle pool solubilized with nonionic detergent from rotavirus V100S-infected MA-104 cells. They do not, however, exclude such a role for a possible cytoskeleton-associated SS particle pool. Gallegos and Patton (17), on the other hand, observed a decrease in radioactivity of SS particles with increasing chase period, but they did not investigate the radioactivity in DS particles with increasing chase period. Contrary to the findings of Gallegos and Patton (17), we and Kabencenell et al. (20) could also not demonstrate an association of nonstructural polypeptides like NS35 with intracellular SS particles. Part of the discrepancies might arise from technical reasons, since Gallegos and Patton (17) used a size separation technique and we and Kabencenell et al. (20) used a density separation technique to isolate intracellular rotaviral particles.

The idea of immunizing animals with empty capsids is not new (35). We have demonstrated that this idea can also be applied to large farm animals of economic importance. In addition, we have shown that empty capsid is the only subunit vaccine that presents VP7 efficiently to the immune system of cows. This finding contrasts with the substantial increase in neutralizing antibody observed in small laboratory animals immunized with VP7 purified by PAGE or isoelectric focusing (5, 22, 28, 33). One obvious interpretation is that the amounts of purified protein that were sufficient to immunize a small animal are insufficient to elicit an immune response in a cow. It remains to be shown whether empty capsids are an attractive alternative to chemically inactivated rotavirus vaccines in cows (36). Preliminary experiments from our laboratory have shown that empty capsid vaccine increased and prolonged rotavirus-specific immunoglobulin G antibody secretion in milk from dairy cows to a similar extent as did DS virus vaccine.

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LITERATURE CITED