Virus-like Particles as a Rotavirus Subunit Vaccine

M. E. Conner, C. D. Zarley, B. Hu, S. Parsons,
D. Drabinski, S. Greiner, R. Smith, B. Jiang, B. Corsaro,
V. Barniak, H. P. Madore, S. Crawford, and M. K. Estes

Rotavirus subunit vaccines are being evaluated for use in humans. The virus-like particles (VLPs) for these vaccines are produced in insect cells coinfected with combinations of baculovirus recombinants expressing bovine RF VP2 and simian SA11 VP4, VP6, or VP7 rotavirus proteins. VLPs were administered parenterally to mice and rabbits, and the immunogenicity and protective efficacy of the vaccines were evaluated. Rabbits vaccinated with VP2/4/6/7 or VP2/6/7 VLP combinations developed high levels of rotavirus-specific serum antibody and fecal IgG but not fecal IgA. The induction of fecal IgG was associated with total or partial protection from oral challenge with ALA rotavirus. Heterotypic serum and fecal neutralizing antibody was induced in mice vaccinated parenterally with G1 VP2/6/7 or VP2/4/6/7 VLPs. VLPs were highly immunogenic when administered in QS21 adjuvant, inducing serum neutralizing antibody titers comparable to those induced by SA11 virus. VLPs are effective immunogens when administered parenterally and may be an effective subunit vaccine.

Rotavirus is a major cause of diarrhea in young children and animals worldwide [1]. Virtually all children are infected with the virus by the time they are 5 years old. The disease can cause a severe dehydrating diarrhea, which results in ~1 million deaths in children annually, primarily in developing countries; thus, development of safe effective vaccines against rotavirus is an international priority, and efforts have focused on live oral attenuated vaccines [1]. The multivalent human-animal reassortant vaccines currently in field trials have had ~70% efficacy against severe disease when tested in developed countries [2–4].

The development of a rotavirus subunit vaccine is being investigated. Rotavirus genes encoding the rotavirus structural proteins are cloned in baculovirus, and the recombinant rotavirus proteins are coexpressed in the baculovirus expression system [5–7]. The expressed rotavirus proteins self-assemble to form virus-like particles (VLPs). Coexpression of different combinations of structural proteins results in formation of VLPs of different protein compositions [5]. Recent evidence that parenteral administration of live or inactivated rotavirus in rabbits results in total protection from homologous oral rotavirus challenge prompted us to evaluate this alternative route of vaccination with VLPs [8]. The immunogenicity and protective efficacy of parenterally administered VLPs of different compositions (VP6/7, VP2/6/7, and VP2/4/6/7) and in different adjuvants (AlOH and QS21) are being evaluated in mice and rabbits.

Materials and Methods and Results

Viruses and cells. The rotavirus strains used in plaque reduction, microneutralization assays, and ELISAs included ALA, Wa, S2, St. Thomas, and SA11. All virus strains were cultivated and plaque purified in MA104 cells [9]. Rotaviruses have a dual serotype specificity based on the two outer capsid proteins VP4 and VP7 that independently elicit neutralizing antibody (NA). VP4 serotypes are designated by P, for protease-sensitive protein. VP4 P types have been defined by serology and sequence analysis. VP4 P genotypes defined by sequence analysis are designated by a number in brackets [10]. VP4 serotypes and genotypes are not numbered identically. The serotype or genotype specificity of the viruses used in this study are ALA (P[14], G3) (Conner ME, unpublished data), SA11 (P[2], G3), Wa (P[1A], G2), S2 (P1B[4], G2), and St. Thomas (P2A[6], G4) [10].

Production of VLPs. VLPs of differing compositions were produced by coinfection of Sf9 cells (at 0.2 or 5 pfu/cell) with different combinations of baculovirus recombinants [5]. The baculovirus recombinants used to produce the VLPs coded for bovine VP2 (RF) or VP6 (C486); simian VP6, P[2] VP4, or G3 VP7 (all SA11); or human G1 VP7 (8697) rotaviruses. VLPs were purified by cesium chloride density-gradient purification or by use of 40% sucrose cushions. They were characterized by biochemical analysis (for purity, protein concentration, and protein composition), electron microscopy (for integrity), ELISA (for VP7 concentration), and limulus amebocyte lysate assay (for endotoxin levels) (figure 1). The serotype of the G1 and G3 VP2/6/7 and VP2/4/6/7 VLPs was confirmed by immunoelectron microscopy with gold-labeled monoclonal antibody probes and with a monoclonal antibody-based serotyping ELISA [5] (Crawford S, unpublished data).

Antigen and antibody assays. Virus and antigen excretion in rabbits was measured by plaque infectivity assays and ELISAs,
Preparation of Rotavirus VLPs

Individual Structural Rotavirus Genes Cloned and Expressed in Baculovirus Infected Sf9 Cells

Purification of VLPs (CsCl or Sucrose)

Evaluate Immunogenicity and Protection

<table>
<thead>
<tr>
<th>Purity SDS PAGE</th>
<th>Composition Western Blot</th>
<th>Integrity Electron Microscopy</th>
<th>Protein Concentration BioRad Protein Assay Nitrogen Analysis</th>
<th>VP7 Concentration ELISA</th>
<th>Endotoxin LAL Assay</th>
</tr>
</thead>
</table>

Figure 1. Outline of production, characterization, immunogenicity, and protective efficacy of rotavirus VLPs. LAL, limulus amebocyte lysate assay. BioRad, Bio-Rad Laboratories (Richmond, CA).

respectively [8, 9, 11]. The relative concentration of VP7 in VLPs and SA11 virus was measured by ELISA (Hu B, unpublished data).

NAs were measured by microneutralization assay [12] or by plaque reduction [9, 11, 13]. Isotype-specific and total antibody (IgA, IgM, and IgG) titers were measured in ELISAs [5, 8, 11, 14].

Immunogenicity and protection studies in mice and rabbits.

Groups of 5 or 6 rotavirus-seronegative CD-1 mice (Charles River, Portage, MI) were intramuscularly (im) vaccinated two times (days 0 and 14) with SA11 rotavirus, VP6/7 or VP2/6/7 G3 VLPs (0.2–20 μg), or buffer in either Freund’s adjuvant, QS21 (a purified triterpene glycoside derivative from the bark of Quillaja saponaria), or AIOH. Blood samples were obtained from mice before and 14 and 28 days after vaccination, and mice were exsanguinated 42 days after vaccination. Mice vaccinated with G1 and G3 VP2/6/7 and VP2/4/6/7 VLPs were vaccinated a third time at day 61. Fecal samples were collected from these mice at 61 and 75 days and assayed for rotavirus-specific total antibody and IgG isotype-specific antibody.

Ten rabbits were im vaccinated twice with 10 or 20 μg of VP2/4/6/7 VLPs or buffer in either Freund’s adjuvant or aluminum phosphate. About 1 month after the second vaccine, rabbits were challenged orally with 10⁵ pfu of ALA (G3) rabbit rotavirus. Serum and fecal samples for antibody assessment were collected before and after vaccination and after challenge at ~2-week intervals. Fecal samples were collected 0–14 days after challenge and assessed for virus or antigen excretion.

Parenteral administration of VLPs in rabbits. We had shown previously that rabbits parenterally vaccinated two times with live or inactivated SA11 (P[2], G3) virus in Freund’s adjuvant or aluminum phosphate were totally protected (no virus or antigen excretion) against ALA (P[14], G3) challenge [8]. Protection was associated with development of rotavirus-specific IgG in the intestine. No rotavirus-specific IgA was detected in the intestine on the day of challenge, but low titers were detected after challenge. To determine if a nonreplicating subunit vaccine also could induce homotypic (G3) protection from viral challenge, rabbits were vaccinated parenterally with G3 VP2/4/6/7 VLPs (figure 2). All rabbits developed rotavirus-specific IgG but not IgA antibody titers in the intestine by the day of challenge and were totally or partly protected from ALA rotavirus challenge. Partial protection from viral challenge was defined as significantly reduced viral or antigen shedding compared with shedding in mock-vaccinated (PBS) controls. Rabbits im vaccinated twice with VP2/6/7 VLPs in QS21 responded similarly. Serologic antibody titers were equivalent to those induced by live or inactivated SA11. The rabbits also developed rotavirus-specific IgG but not IgA antibody titers in the intestine and were partially protected from viral challenge.

Induction of heterotypic NA by G1 VP2/6/7 and VP2/4/6/7 VLPs. We have previously shown that G3 VP2/4/6/7 VLPs are highly immunogenic in mice, inducing high levels of NA [5]. In addition, we demonstrated that chimeric VLPs with both a G1 and a G3 VP7 displayed on their surface induce NA to both G1 (Wa) and G3 (SA11) viruses. In these experiments, we obtained preliminary data indicating that VP2/6/7 VLPs displaying only G1 VP7 on
their surface also induced NA to G3 (SA11) virus. To confirm these results and to determine if the heterotypic NAs were induced because VP4 was not present in the VLPs, mice were immunized with VP2/6/7 or VP2/4/6/7 G1 or G3 VLPs [15]. Before and 42 days after vaccination, serum samples were tested for NA to G1 (Wa), G2 (S2), G3 (SA11), and G4 (St. Thomas) viruses. Mice vaccinated with G1 VLPs, either VP2/6/7 or VP2/4/6/7, developed NA to G1 and G3 viruses but not to G2 or G4 viruses. Heterotypic fecal NA to G3 virus also was detected in mice vaccinated with G1 VLPs. The fecal samples have not yet been tested against G1, G2, or G4 viruses. Mice vaccinated with G3 VLPs developed intestinal homotypic NA to G3 virus.

Stability, homogeneity, and immunogenicity of VP6/7 and VP2/6/7 VLPs. Development of a stable, homogeneous, and immunogenic subunit vaccine containing the least number of coexpressed proteins in the VLPs is preferable for ease and cost of production. We have previously reported that VLPs containing bovine VP2 and simian VP6 and VP7 were more stable [5]. The stability and homogeneity of VLPs containing bovine VP6 and simian VP7 with or without bovine VP2 was assessed by electron microscopy. Following purification, the VP2/6/7 VLPs were more homogeneous than VP6/7 VLPs, and more were intact (figure 3). The VP2/6/7 VLPs also maintained greater particle integrity after repeated ultracentrifugation.

The immunogenicity of VP6/7 and VP2/6/7 VLPs and SA11 given parenterally was compared in CD-1 mice. The immunogens were administered twice in either ALOH or QS21 adjuvant. The serum antibody responses were compared 42 days after the first vaccination by both ELISA and microneutralization assay (table 1). Both VLP formulations in either adjuvant were immunogenic in mice. The highest antibody responses were in mice immunized with QS21, irrespective of the type of VLPs administered. Both VP6/7 and VP2/6/7 in QS21 induced antibody responses (ELISA and neutralizing) comparable to those in mice immunized with SA11 in QS21. In dose-response studies with VP2/6/7 VLPs, the magnitude of the immune response varied with the dose of VLPs and QS21 (data not shown).

Discussion

VLPs may provide a safe and efficacious alternative or complement to live rotavirus vaccines for both human and veterinary use [16]. We have shown that VLPs administered parenterally to rabbits and mice are highly immunogenic: all formulations of VLPs induced high levels of NA and total immunoglobulin antibody in serum and intestine. In rabbits, VP2/4/6/7 or VP2/6/7 VLPs induced rotavirus-specific IgG but not IgA antibody in the intestine that was associated with protection: Vaccinated rabbits were totally or partly protected from homotypic (G3) rotavirus challenge. These data support results we obtained previously with parenterally administered live or inactivated SA11, which induced total protection in rabbits against rotavirus challenge [8]. The lower level of protection following vaccination with VLPs correlated with titers of fecal IgG that were lower than those in rabbits vaccinated with SA11 virus.

We have used a stringent measure of protective efficacy in our studies—protection from infection. In children, the protective efficacy of the live oral vaccines being field tested is based on protection from severe disease, a much less stringent measure of protective efficacy. The protective efficacy of VLPs against clinical disease needs to be evaluated, but currently, neither the rabbit nor the mouse can be used to evaluate clinical
JID 1996;174 (Suppl I ) VLPs as a Rotavirus Subunit Vaccine

Figure 3. Electron micrograph of rota virus VP6/7 and VP2/6/7 VLPs. VP6/7 VLPs were less stable (more broken VLPs) and less homogeneous than VP2/6/7 VLPs.

Table 1. Immunogenicity of VP6/7 and VP2/6/7 virus-like particles (VLPs) and SA11 rotavirus parenterally administered in AIOH or QS21 adjuvant to CD-1 mice.

<table>
<thead>
<tr>
<th>Adjuvant, vaccine*</th>
<th>ELISA</th>
<th>Neutralization</th>
</tr>
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<tbody>
<tr>
<td>AIOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP6/7</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>VP2/6/7</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>SA11</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Buffer</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>QS21</td>
<td></td>
<td></td>
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<tr>
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<td>3+</td>
</tr>
<tr>
<td>SA11</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Buffer</td>
<td>Negative</td>
<td>Negative</td>
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</tbody>
</table>

NOTE. Data are graded responses, with 3+ being highest antibody titer. Vaccines were given twice (days 0 and 14). QS21 = purified triterpene glycoside derivative from bark of *Quillaja saponaria*.

* VLP-vaccinated mice received equivalent amounts of VP7. Total protein concentration of vaccines was 10 µg of VP6/7 VLPs, 15 µg of VP2/6/7, and 5 µg of SA11.

An effective vaccine in children will need to induce an immune response to multiple rotavirus serotypes [1]. For the live oral vaccines, multivalent formulations containing the four most prevalent G types (1-4) are ~70% efficacious against severe disease [2-4]. However, with any vaccine (live attenu-
ated or subunit), the cost of producing multivalent formulations increases as the number of components increases. The induction of NAs to both G1 and G3 viruses with G1 VP2/6/7 and VP2/4/6/7 VLPs indicates that it may be possible to reduce the number of G types incorporated into a subunit vaccine and that the incorporation of VP4 may not be necessary. Therefore, it may be feasible to produce VLP subunit vaccines with a limited number of types and proteins, thereby reducing production costs. Induction of heterotypic NA by the G1 VP7 may be the result of similar amino acid sequences in the C regions of human 8697 G1 and G3 rotaviruses [20]. The protective efficacy of the G1 VLPs to homotypic and heterotypic challenge needs to be evaluated.

Although VP6/7 VLPs were immunogenic in mice, the relative instability and lack of homogeneity of these particles make them an unviable vaccine candidate. However, the VP2/6/7 VLPs are stable and homogeneous and induce high levels of NA and total antibody in both mice and rabbits. In addition, the VP2/6/7 VLPs induced protection in rabbits, indicating that VP4 may not be a required component of a VLP formulation.

On the basis of protection studies in rabbits and immunogenicity studies in mice and cows [16, 21], VLPs show promise as a subunit vaccine for both humans and animals. Our results clearly indicate that alternative immunization routes (e.g., im) may be effective and warrant further investigation. Incorporation of a parenterally administered rotavirus vaccine into pediatric parenteral formulations already being given to children may be possible. Rotavirus VLPs also are being evaluated as oral immunogens, and preliminary results in mice indicate that VLPs may provide a safe and effective oral subunit vaccine.

References