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Intranasal Immunization of Mice with Group B Streptococcal Protein Rib and Cholera Toxin B Subunit Confers Protection against Lethal Infection

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Several approaches have been tried in an attempt to design an efficient vaccine against group B streptococcal (GBS) disease (7), and possible vaccine components include capsular polysaccharides and cell surface proteins of GBS (2, 8, 9, 12, 16, 17, 20, 27). Mucosal immunization is an attractive strategy, since it is noninvasive and has the potential to induce both systemic and local immune responses (3, 14, 22). Indeed, it should be advantageous to use a GBS vaccine that not only induces protective immunoglobulin G (IgG) antibodies that can be transplacentally transferred to the fetus but also induces mucosal antibodies that prevent genital colonization of the mother and thereby prevent transfer of the bacteria to the fetus.

Some human and animal studies have focused on antibodies to GBS at the female genital mucosa (8–12, 24). Although, the role of these local antibodies in the pathogenesis of GBS infection is not clear, it seems reasonable to assume that they may to some extent protect against colonization with GBS (10).

It has been reported that mucosal immunization with GBS polysaccharides or inactivated GBS bacteria induces systemic and local antibody responses in mice (8, 9, 12, 24). However, there is no previous report on mucosal immunization with purified GBS proteins.

Earlier studies have shown that parenterally administered cell surface proteins of GBS elicit a systemic IgG response and confer protection against experimental GBS infection (1, 6, 17, 18, 20). This made it of interest to examine if these proteins could also be used in a mucosal GBS vaccine. In the present study, Rib, a well-characterized GBS cell surface protein that is expressed by many strains causing invasive neonatal infection (17, 25, 26), was combined with recombinant cholera toxin B subunit (CTB) and administered intranasally (i.n.) to mice. The systemic and local IgG and IgA responses were examined. In addition, the protective capacity of this mucosal vaccination was evaluated by lethal intraperitoneal (i.p.) challenge with GBS.

Preparation of conjugate vaccine. The Rib protein was isolated from the high-virulence type III strain BM110 (21, 25) by several purification steps and was free of contaminating polysaccharides (17, 25). Recombinant CTB was purified from Vibrio cholerae strain 358 (19). The Rib protein was conjugated to CTB using N-succinimimidyl 3-(2-pyridyldithio)propioninate (SPDP; Pharmacia AB, Uppsala, Sweden) as a bifunctional coupling reagent. SPDP was added to both proteins according to the manufacturer's instructions, using pH 8.5 for Rib and pH 7.5 for CTB. A similar method has been used for coupling polysaccharides to CTB and is described in detail elsewhere (4, 5). A 10-fold molar excess was used for Rib, and a 3-fold molar excess was used for CTB. The 2-pyridyl disulfide-containing Rib was then reduced using 50 mM dithiothreitol, and the excess was used for CTB. The 2-pyridyl disulfide-containing Rib was then reduced using 50 mM dithiothreitol, and the excess 2-pyridyl disulfide and dithiothreitol was removed using a Sephadex G25 prepacked PD10 column (Pharmacia AB, Uppsala, Sweden). The two derivatized proteins were mixed and allowed to react overnight at room temperature. The conjugate was purified using a Superdex 200 10/30 column connected to a BioLogic WorkStation (Bio-Rad). The fractions containing the highest-molecular-weight material were collected, pooled, and concentrated using an Amicon stirred concentration cell (Amicon). The conjugate was tested by enzyme-linked immunosorbent assay (ELISA), and both CTB and Rib were detected. The Lowry protein determination assay was used to measure the total protein content of the conjugate. However, the exact proportion of each protein could not be determined. The conjugate was stored at 4°C with 0.005% Merthiolat (Kebo AB, Stockholm, Sweden) as a preservative.

Immunizations and antibody responses. Five groups with four 10-week-old C3H/HeN female mice (Charles River, Sulzfeld, Germany) in each group were immunized i.n. with either of the following vaccines: protein Rib conjugated to CTB (Rib-CTB) (8 μg), Rib mixed with CTB (Rib+CTB) (4 μg plus 4 μg), Rib alone (Rib) (4 μg), CTB alone (CTB) (4 μg), or phosphate-buffered saline (PBS) three times 2 weeks apart. One group with four mice received 5 μg of Rib subcutaneously (s.c.) and 4 weeks later a booster of 2.5 μg s.c. The antigens were diluted in PBS to a volume of 25 μl for i.n. administration and 150 μl for s.c. administration, respectively. The mice were lightly anesthetized with methoxyflurane (Methofane) (Mallin-
TABLE 1. Titers of Rib-specific IgG in serum after immunization with different vaccine compositions

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Route of immunization</th>
<th>Titer of IgG, geometric mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib-CTB</td>
<td>i.n.</td>
<td>6,959 (1,772–27,258)</td>
</tr>
<tr>
<td>Rib + CTB</td>
<td>i.n.</td>
<td>10,342 (6,288–14,998)</td>
</tr>
<tr>
<td>Rib</td>
<td>i.n.</td>
<td>86 (40–261)</td>
</tr>
<tr>
<td>CTB</td>
<td>i.n.</td>
<td>4 (≤3–11)</td>
</tr>
<tr>
<td>Rib s.c.</td>
<td></td>
<td>2,243 (640–4,030)</td>
</tr>
</tbody>
</table>

* a Mice (four per group) were immunized thrice with Rib-CTB, Rib + CTB, Rib, or CTB i.n. or twice with Rib s.c.
  b The titers of antibody were presented as the antilog of the geometric mean and range. Titers of ≤3 were not detected. Background values from mice sham immunized with PBS are subtracted.

TABLE 2. Titers of Rib-specific IgA in serum and genital tissue extract after immunization with different vaccine compositions

<table>
<thead>
<tr>
<th>Vaccine tissue</th>
<th>Route of immunization</th>
<th>Titer of IgA, geometric mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib-CTB</td>
<td>i.n.</td>
<td>53 (36–87)*</td>
</tr>
<tr>
<td>Rib + CTB</td>
<td>i.n.</td>
<td>8 (4–18)</td>
</tr>
<tr>
<td>Rib i.n.</td>
<td>≤3</td>
<td>≤3</td>
</tr>
<tr>
<td>CTB i.n.</td>
<td>≤3</td>
<td>≤3</td>
</tr>
<tr>
<td>Rib s.c.</td>
<td>4 (≤3–5)*</td>
<td>4 (≤3–9)</td>
</tr>
</tbody>
</table>

* a Mice (four per group) were immunized thrice with Rib-CTB, Rib + CTB, Rib or CTB i.n. or twice with Rib s.c. The genital tissue was extracted with 2% saponin-PBS.
  b The titers of antibody were presented as the antilog of the geometric mean and range. Titers of ≤3 were not detected.
  c Only three sera were available for analysis of IgA.

Intranasal immunization of mice with Rib-CTB or Rib + CTB elicited significant levels of serum IgG to protein Rib (Table 1). In contrast, the IgG response induced by i.n. immunization with Rib alone was approximately 100 times lower (Table 1). In agreement with previous studies (17, 18), s.c. immunization with protein Rib elicited a systemic Rib-specific IgG response (Table 1).

Rib-specific IgA antibodies were found in serum and in genital tissue extracts from mice i.n. immunized with Rib-CTB. Low levels of IgA were also detected in serum from mice that received Rib + CTB (Table 2). In contrast, Rib given i.n. without CTB did not result in any measurable IgA response. The method used in this study for measuring local antibodies in mucosal tissue has previously been evaluated regarding the proportion of serum antibodies from blood retained in the tissue extracts. The proportion of serum IgA antibodies in genital tissue extracts in that control experiment was approximately 2% (13). We compared the titers of IgA in the genital tissue extracts after i.n. immunization with Rib-CTB with the corresponding titers of IgA in serum, and we found that most of the IgA antibodies in the genital tissue appeared to be locally produced, according to the observations made in the previous control experiment referred to above (13).

Protection against lethal GBS infection. Groups of 15 mice were vaccinated with Rib-CTB i.n., Rib + CTB i.n., Rib s.c., or PBS i.n., as described above. Two weeks after the booster mice were challenged i.p. with a ~90% lethal dose of log-phase bacteria (BM110) diluted in 0.5 ml of Todd-Hewitt broth. Deaths were recorded daily for 7 days (17). Vaccine efficacy (or protective efficacy) was calculated by the following formula: (mortality in nonvaccinated – mortality in vaccinated)/(mortality in nonvaccinated). Statistical significance was estimated by Fisher’s exact test and confidence intervals (CI). The confidence level was set at 95%. A P value of less than 0.05 was considered statistically significant.

Immunization with CTB–Rib or CTB + Rib provided protection against lethal infection with the GBS type III strain BM110 expressing the Rib protein (Fig. 1). Ten of fifteen mice vaccinated with Rib-CTB i.n. and 12 of 15 mice vaccinated with Rib + CTB survived a challenge with a lethal i.p. dose of GBS. The protective efficacy for Rib-CTB was 55% (CI, 37 to 73; P = 0.03) and 73% (CI, 57 to 89; P = 0.005) for Rib + CTB. In agreement with previous reports (17, 18), s.c. vaccination with the Rib protein protected against lethal GBS infection. We observed, however, that even though the amount of the Rib protein used for s.c. immunization was reduced and was given without adjuvant, it still induced sufficient immunity to confer
complete protection against lethal GBS infection ($P = 0.000002$) (Fig. 1).

Conclusions. Intranasal immunization of mice with the Rib protein and CTB seems to induce systemic and local antibody responses and to confer protective immunity against GBS infection. The titers of IgG induced by immunization with Rib alone (●) and s.c. immunization with Rib alone (●) were challenged i.p. with a ~90% lethal dose of GBS strain BM110. Deaths were recorded daily for 7 days. Fisher’s exact test was used to calculate $P$ values.

The majority of clinically important GBS strains express either of the two proteins Rib or α (17, 25). Previously, we have shown that it is possible to include these two GBS cell surface proteins in a vaccine and elicit protective immunity in mice without any sign of immunogenic competition between the vaccine antigens (18). We conclude that i.n. immunization with a vaccine containing cell surface proteins from GBS strains that are prevalent among pregnant women may be an alternative strategy in the ongoing efforts to design an efficient vaccine against neonatal GBS disease.

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