Plasmid-Cured *Salmonella enteritidis* AL1192 as a Candidate for a Live Vaccine

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We report the immunizing capacity of *Salmonella enteritidis* AL1192, a strain that has been cured of a 36-megadalton plasmid, to protect ddY mice against subsequent challenge with virulent salmonellas. This strain, which was given subcutaneously at a dose of $10^6$ organisms, provided significant protection against oral, subcutaneous, or intraperitoneal challenge by virulent wild-type strains of not only *S. enteritidis*, but also *S. dublin*, *S. naestved*, and *S. typhimurium*.

Recently, three reports (3, 5, 9) showed that virulence was associated with the presence of a plasmid in *Salmonella typhimurium*, *S. dublin*, and *S. enteritidis*, the principal causes of animal and human salmonellosis. Because curing the plasmid of these three serovars resulted in a decrease in virulence for mice, it is reasonable to assume that plasmid-cured derivatives of these strains may be effective live vaccines. Therefore, we tested the potential of a plasmid-cured derivative of *S. enteritidis* as an effective live vaccine.

Virulent *S. enteritidis* AL1190, which carries a 36-megadalton plasmid, was isolated from the spleen of a dairy cow; AL1192 is a plasmid-cured derivative of AL1190 (5). The 50% lethal doses (LD$_{50}$) of these two strains in 5-week-old ddY mice were $10^4.51$ and $10^7.85$ bacteria on subcutaneous administration and $10^8.71$ and $>10^8$ on oral administration, respectively (5). Figure 1 shows the viable counts of *S. enteritidis* AL1190 and AL1192 organisms in the spleens of C57BL mice (salmonella-susceptible inbred line [6]) up to 14 days after the administration of various doses of bacteria.

We tested the ability of AL1192 to protect against subsequent challenge with AL1190. The mice (ddY 5-week-old males) were subcutaneously given $10^6$ to $10^7$ AL1192 bacteria and challenged 2 weeks later with graded doses of virulent AL1190. Deaths were recorded daily for 14 days. AL1192, given subcutaneously at a dose of $10^6$, gave adequate protection against oral and subcutaneous challenge, whereas almost all nonimmunized control mice were dead by day 14 (Table 1). Thus, subcutaneous injection of $10^6$ live AL1192 bacteria protected mice challenged with a lethal dose. Moreover, AL1192, given orally at a dose of $10^6$, also gave protection against subcutaneous challenge with $10^6$ LD$_{50}$ of AL1190 (data not shown). Hoiseth and Stocker (2) reported that aromatic-dependent *S. typhimurium* completely protected mice against oral or peritoneal challenge of virulent *S. typhimurium*. Our results are similar, although different *Salmonella* serovars were used.

We next examined the ability of AL1192 to protect against subsequent challenge with another wild-type strain of *S. enteritidis* and several wild-type strains of *S. dublin*, *S. naestved*, and *S. typhimurium*. Sources and O-antigenic formula of these challenge strains are shown in Table 2. AL1192 that was given subcutaneously at a dose of $10^6$ provided significant protection against oral, subcutaneous, and intraperitoneal challenge by virulent wild-type strains of not only *S. enteritidis*, but also *S. dublin*, *S. naestved*, and *S. typhimurium* (Table 2). Although there were minor differences in level of protection according to the challenge strain or route, in most cases AL1192 protected mice against $10^2$ to $10^3$ LD$_{50}$ challenge regardless of challenge strain. Immunoization of mice with plasmid-cured *S. enteritidis* AL1192 organisms induced not only protection against *S. dublin* and *S. naestved* having the same O-antigenic formula as *S. enteritidis*, but also cross-protection against *S. typhimurium* strains with different antigenic formulas. Smith et al. (7, 8) reported that an aromatic-dependent live vaccine of *S. typhimurium* protected calves against challenge with virulent *S. dublin* and vice versa. According to their reports, the protection given by one serovar against challenge by other serovars might not be adequately explained by O-specific humoral immunity, although the O-antigenic structures of

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FIG. 1. Log viable count of *S. enteritidis* AL1190 and AL1192 organisms in spleens of C57BL mice.
TABLE 1. Challenge by subcutaneous or oral administration of virulent S. enteritidis AL1190 to ddY mice immunized by subcutaneous injection of S. enteritidis AL1192

<table>
<thead>
<tr>
<th>AL1192 immunization dose</th>
<th>Deaths/no. tested at AL1190 challenge dose (oral administration):</th>
<th>AL1192 immunization dose</th>
<th>Deaths/no. tested at AL1190 challenge dose (subcutaneous administration):</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 x 10^7</td>
<td>0/5</td>
<td>3.9 x 10^6</td>
<td>1/5</td>
</tr>
<tr>
<td>6.1 x 10^8</td>
<td>0/5</td>
<td>3.9 x 10^6</td>
<td>4/5</td>
</tr>
<tr>
<td>6.1 x 10^9</td>
<td>1/5</td>
<td></td>
<td>4/5</td>
</tr>
<tr>
<td>6.1 x 10^10</td>
<td>2/5</td>
<td>2/5</td>
<td>2/5</td>
</tr>
<tr>
<td>Control</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

TABLE 2. Challenge of virulent salmonellas to ddY mice immunized by subcutaneous injection of 10^8 S. enteritidis AL1192 bacteria

<table>
<thead>
<tr>
<th>Challenge strain</th>
<th>O-antigen</th>
<th>Source</th>
<th>Route</th>
<th>LD50 (loglo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. enteritidis</td>
<td>1.9,12</td>
<td>Spleen (calf)</td>
<td>i.p.</td>
<td>7.18</td>
</tr>
<tr>
<td>AL1190</td>
<td></td>
<td></td>
<td>s.c.</td>
<td>6.63</td>
</tr>
<tr>
<td>L-174</td>
<td>1.9,12</td>
<td>Kidney (calf)</td>
<td>p.o.</td>
<td>9.85</td>
</tr>
<tr>
<td>S. dublin</td>
<td>1.9,12</td>
<td>Liver (calf)</td>
<td>s.c.</td>
<td>9.72</td>
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<tr>
<td>L-107</td>
<td></td>
<td></td>
<td></td>
<td>6.52</td>
</tr>
<tr>
<td>S. naestved</td>
<td>1.9,12</td>
<td>Spleen (calf)</td>
<td>i.p.</td>
<td>8.18</td>
</tr>
<tr>
<td>L-595</td>
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<td></td>
<td>s.c.</td>
<td>5.18</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>4.5,12</td>
<td>Lymph node (calf)</td>
<td>i.p.</td>
<td>4.86</td>
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<tr>
<td>L-535</td>
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<td></td>
<td>s.c.</td>
<td>7.20</td>
</tr>
<tr>
<td>L-545</td>
<td>1.4,12</td>
<td>Feces (calf)</td>
<td>i.p.</td>
<td>6.18</td>
</tr>
</tbody>
</table>

*LITERATURE CITED*


