Oral Vaccination of Weaned Rabbits against Enteropathogenic 
*Escherichia coli*-Like *E. coli* O103 Infection: Use of 
Heterologous Strains Harboring Lipopolysaccharide 
or Adhesin of Pathogenic Strains

ALAIN MILON,1* JÜRGEN ESSLINGER,2 AND ROBERT CAMGUILHEM3

Département de Biologie Moléculaire, Unité Associée de Microbiologie Moléculaire, Institut National de la 
Recherche Agronomique, Unité Associée de Microbiologie Moléculaire, Institut National de la 
Recherche Agronomique, 23, Chemin des Capelles, F-31076 Toulouse Cedex, 
France,1 and Institut für Bakteriologie und Immunologie, Fachbereich Veterinärmedizin, 
Justus-Liebig-Universität, D-6300 Giessen, Germany2

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To test the importance of lipopolysaccharide (LPS) and adhesin as major antigens in vaccination against 
rabbit enteropathogenic *Escherichia coli* (EPEC)-like *E. coli* O103 infection, we used two nonpathogenic 
wild-type strains to immunize rabbits at weaning. One of these strains (C127) harbors the O103 LPS but does not 
express the 32,000-molecular-weight adhesin that characterizes the highly pathogenic O103 strains. The 
other (C6) belongs to the O128 serogroup, which does not cross-react with the O103 serogroup, but expresses 
the adhesin. These strains were administered orally, either live or after Formalin inactivation. After 
vaccination, the animals were challenged with highly pathogenic O103 strain B10. Compared with rabbits 
vaccinated with the Formalin-killed homologous strain, rabbits vaccinated with killed C127 or C6 showed 
partial but significant protection. When given live, these strains colonized more or less heavily the digestive 
tract of the animals and provided nearly complete (C127) or complete (C6) protection against challenge. They 
induced a quick local immune response, as judged by fecal immunoglobulin A anti-LPS kinetics. Furthermore, 
strain C6 induced an ecological effect of "resistance to colonization" against challenge strain B10. This effect 
may have been due to the adhesin that is shared by both strains and to the production of a colicin. Strain C6 
could inhibit in vitro the growth of highly pathogenic O103 strains. On the whole, our results show that 
adhesins and LPS are important, although probably not exclusive, protection-inducing components in rabbit 
EPEC-like colibacillosis and provide insight into possible protection of rabbits against EPEC-like *E. coli* 
infection with live strains.

*Escherichia coli* enteritis is frequent in rabbit-fattening 
units of Western Europe. In France, this disease is consid-
ered a major problem of rabbit breeding, especially when 
it involves highly pathogenic strains belonging to the O103 
serogroup and rhamnose-negative biotypes (5). These strains 
cause diarrhea with high mortality (up to 30%) and 
considerable growth retardation, leading to substantial economic 
losses. O103, rhamnose-negative strains are currently 
considered enteropathogenic *E. coli*-like strains (i.e., strains 
similar to human enteropathogenic *E. coli* strains, as defined 
by Levine [19]) or attaching-effacing *E. coli*-like strains (28, 
31, 42), by virtue of their ability to colonize heavily the distal 
regions of the intestinal tract, with typical cuplike adhesion 
to enterocytes in vivo, as seen by electron microscopy (13). 
An in vitro study of the adhesion of O103 strains to rabbit 
testinal villi and to HeLa cells recently led to the discovery of 
a 32,000-molecular-weight protein (32K protein or ad-
hesin) that is prominent in surface extracts of the bacteria 
and is clearly implicated in attachment mechanisms (27). 
This protein inhibits the adhesion of bacterial cells in a 
competitive way, and specific antibodies to the 32K protein 
neutralize the ability of the bacteria to adhere in vitro to cells 
(27).

We have tried different vaccination protocols to protect 
animals during their economical life (i.e., between weaning at about 30 days of age and slaughter at between 9 and 11 
weeks of age). Successive trials have led to different conclu-
sions. (i) Vaccination by the parenteral route at weaning is 
ineffective in protecting the animals against a challenge with 
virulent strain B10 (O103, rhamnose negative) (3). (ii) Oral 
vaccination of does induces local responses, with the pro-
duction of secretory specific immunoglobulin A (IgA) in 
milk, but is ineffective in protecting the litter after weaning 
(25, 26). (iii) Successful protection of weaned rabbits may 
be achieved by oral vaccination at weaning with Formalin-
killed whole cells, but the schedule and doses to be used are 
difficult to apply in practice: the initial schedule required 10 
daily doses of 4 × 10⁶ CFU of strain B10 grown in Trypticase 
soy broth (6); this schedule was shortened to 4 daily doses 
when the vaccine strain was grown in Penassay broth 
(antibiotic medium 3; Difco, Detroit, Mich.), a medium that 
favors the expression of the 32K adhesin (26). All these 
 studies were carried out under homologous conditions: the 
vaccines were prepared with inactivated strain B10, which 
was then given live as a challenge.

Results of vaccination trials carried out under heterolo-
gous conditions are presented here. Two strains were used 
as vaccines, either after Formalin inactivation or live. These 
strains express either lipopolysaccharide (LPS) or adhesin 
of pathogenic O103 strains. The animals were challenged 
with our reference pathogenic O103 strain, B10. These protocols 
provide an insight into the relative importance of LPS and 
adhesins as protective antigens and may be considered a first 
approach to the vaccination of weaned rabbits against O103
TABLE 1. E. coli strains used*  

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serovar; biotype*</th>
<th>32K adhesin*</th>
<th>Experimental pathogenicity*</th>
<th>Experimental use</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10</td>
<td>O103:H2:K−; Rha−</td>
<td>+</td>
<td>+</td>
<td>Challenge (all groups); Formalin-killed vaccine (KB10); colicin target</td>
</tr>
<tr>
<td>C55, C70, C148, and C199</td>
<td>O103:H2:K−; Rha−</td>
<td>+</td>
<td>+</td>
<td>Colicin targets</td>
</tr>
<tr>
<td>C127</td>
<td>O103:H2:K?; Rha*</td>
<td>−</td>
<td>−</td>
<td>Formalin-killed vaccine (KC127); live vaccine (LC127); colicin production</td>
</tr>
<tr>
<td>C124</td>
<td>O103:H2:K?; Rha*</td>
<td>−</td>
<td>−</td>
<td>Colicin production</td>
</tr>
<tr>
<td>C6</td>
<td>O128:H2:K?; Rha*</td>
<td>+</td>
<td>−</td>
<td>Formalin-killed vaccine (KC6); live vaccine (LC6); colicin production</td>
</tr>
<tr>
<td>C104</td>
<td>O128:H2:K?; Rha*</td>
<td>+</td>
<td>−</td>
<td>Colicin production</td>
</tr>
</tbody>
</table>

* All the strains were isolated from weaned commercial rabbits.
* As described in reference 5. The rhamnose-negative character is correlated with pathogenicity in weaned rabbit E. coli isolates.
* As described in reference 27. +, presence; −, absence.

As described in reference 5. Pathogenicity was checked by oral inoculation of 10⁷ CFU of the strains to 35-day-old rabbits. Diarrhea and mortality were checked over a 30-day period. +, high rate of diarrhea and mortality; −, no diarrhea or mortality.

Materials and Methods

E. coli strains. All the strains used were rabbit field isolates from farms at which diarrheic episodes had occurred (Table 1). Challenge strain B10 has been used since 1986 to reproduce enterocolitis in weaned rabbits (4). It belongs to serovar O103:H2:K− and has a rhamnose-negative biotype; such strains have been shown to be highly pathogenic in weaned rabbits and epidemiologically predominant in France (4, 5). B10 expresses a 32K adhesin, which confers to the bacteria the ability to attach in vitro to intestinal villi of 8-day-old and 6-week-old rabbits and to HeLa 229 cells (diffuse adhesion) (27). This strain was used as an inactivated vaccine (group KB10; see below) and as a challenge strain (all groups; see below). Strains C55, C70, C148, and C199, used in colicin detection, belong to the same serogroup as B10 and have the same properties (5, 27). Vaccine strain C127 belongs to serogroup O103 but is rhamnose positive and was experimentally determined to be nonpathogenic for weaned rabbits; i.e., it induced no diarrhea or mortality upon oral inoculation of 10⁷ CFU (5). In vitro, it adheres to 6-week-old rabbit intestinal villi but not to 8-day-old ones or HeLa cells (26). C124 is a strain analogous to C127. Vaccine strain C6 belongs to serogroup O128 (H?:K?). This serogroup does not cross-react with serogroup O103 (30). Strain C6 is rhamnose positive and was experimentally determined to be nonpathogenic (5) but harbors the same adhesin and has the same in vitro adhesive properties as pathogenic O103 strains (27). Strain C104 has the same properties as strain C6. When cross-tested in Western immunoblotting with rabbit sera raised against Formalin-killed whole cells, B10 and C6 shared mainly the 32K adhesin and 36K and 64K molecules. C127 shared with B10 the two last bands and several others, including the ladder-shaped bands typical for LPS, but not the adhesin band.

Animals. One hundred thirty six New Zealand male rabbits (Institut National de la Recherche Agronomique strain 1066 or 1077) were used in the experiments. They were weaned at 28 or 29 days of age and divided into vaccinated or control groups according to litter and body weight (i.e., the litters were split among different groups, and the average weight in each group was approximately the same). They were housed in cages of four animals and fed with a coccidioiastic supplemented feed (Robenidine) and given water ad libitum throughout the experiment.

Vaccination and challenge protocols. Two different vaccine types and protocols were used. (i) Formalin-killed vaccines comprised strain B10 (group KB10), C127 (group KC127), or C6 (group KC6). Vaccines were prepared as described previously (6), except that strains were grown in Penassay broth, which favors the expression of the 32K adhesin (27). Vaccine doses (=4 x 10⁷ CFU ml⁻¹) were given by oral cannula daily for 4 consecutive days. Vaccination was started 1 day after weaning (day 0). On day 9, animals were challenged per os with 2 x 10⁹ live E. coli B10 cells in 2 ml of saline. (ii) Live vaccines comprised strain C127 (group LC127) or C6 (group LC6) cultured in Trypticase soy broth and eventually diluted in saline. Doses of 5 x 10⁹ CFU (group LC127) or 5 x 10⁹ CFU (group LC6) were given per os 1 day after weaning (day 0), and the animals were challenged as described above on day 7.

Before use, Formalin-killed vaccines were checked for the expression of the 32K adhesin by a Western dot blot assay. Fifty microliters (=10⁴ CFU) of inactivated vaccine cultures was vacuum blotted onto a nitrocellulose sheet. The sheet was air dried and treated as already described (27), except that a 1% (vol/vol) concentration of 30% hydrogen peroxide was added to the first wash. Vaccines prepared from strains B10 and C6 showed a strong reaction when tested with an antiserum specific for the 32K adhesin. The C127 vaccine did not react under these conditions.

Control groups did not receive any vaccine and were challenged as described above. After challenge, animals were observed daily for diarrhea and mortality. Individual weight was recorded three times a week. Feces were sampled each week for E. coli enumeration by the MacConkey agar dilution technique. Three or six clones per sample were subcultured and tested with anti-O103 or anti-O128 antiserum by slide agglutination; their ability to ferment rhamnose was tested on phenol red agar plus 1% rhamnose. These tests are especially useful in live vaccination protocols, as they enable vaccine strains (C127: O103, rhamnose positive; C6: O128, rhamnose positive) to be quickly differentiated from the challenge strain (B10: O103, rhamnose negative) or strains from normal flora, which are neither O103 nor O128 and rhamnose positive. We also checked for coccidial oocysts by enumeration in a MacMaster chamber. Postmortem examinations were performed on animals that died on challenge, and the same techniques were used to prepare
cecal contents for *E. coli* enumeration and coccidial oocyst counts to ensure that death was due to the challenge strain.

**Antibody monitoring.** Fresh feces were sampled once a week for anti-LPS IgA. Feces (2 g) were homogenized in 8 ml of phosphate-buffered saline (PBS) containing 0.02% sodium azide (1/5 dilution [wt/vol]). After trituration with glass beads and centrifugation for 20 min at 3,500 × g and 4°C, the supernatant was kept at −20°C until assayed. The O103 and O128 LPSs used as antigens were prepared from strains B10 and C6, respectively, as described by Adams (1). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the LPS preparations did not reveal any trace of protein contamination after silver staining of the gel. The enzyme-linked immunosorbent assay technique used to measure fecal anti-LPS IgA has been described elsewhere (25). In brief, the LPS was adsorbed in excess to the wells of microtiter plates by overnight incubation at 4°C in 0.02 M carbonate buffer (pH 9.6). Samples (100 μl) were incubated at 37°C for 30 min at a final dilution of 1/20 in PBS containing 0.2% (vol/vol) Tween 20 in two wells containing O103 LPS or two wells coated with O128 LPS and one control well. The wells were then washed three times with 300 μl of PBS containing 0.05% (vol/vol) Tween 20 delivered by a Titertek Autodrop (Flow Laboratories). Anti-LPS IgA was detected by sequential incubation for 30 min at 37°C with goat anti-rabbit IgA (alpha-chain specific) (Miles Scientific, Naperville, Ill.) and, after three washes, rabbit anti-goat IgG (heavy- and light-chain specific) conjugated to alkaline phosphatase (Biosys, Compiègne, France). After four final washes, the substrate of the enzyme, p-nitrophenyl phosphate (1 mg/ml) in diethanolamine-HCl buffer (pH 9.8), was added. Plates were incubated at room temperature until the optical densities (OD) at 405 nm in wells containing standard strongly and faintly positive fecal samples reached their usual values. The results were read with a multichannel spectrophotometer (Titertek Multiskan; Flow Laboratories) and expressed as milliunits of specific OD (i.e., mean of OD in test wells minus OD in the control well). The OD measured in the control well never exceeded 50 mU.

**Test for colicinlike activity.** Presumptive tests for colicins active against pathogenic O103 strains were run for strains C127, C124, C6, and C104. Target O103 strains were streaked on Mueller-Hinton agar. After being air dried for 20 min at 37°C, test strains were inoculated as spots on the streaks of target strains. After 18 h at 37°C, colicinlike activity was revealed by inhibition of the growth of target strains around the colonies of test strains.

**RESULTS**

**Morbidity and mortality following challenge.** Clinical results for control (unvaccinated) groups were nearly identical, with high proportions of weight loss (in almost 90% of the animals), diarrhea (in 80 to 90% of the animals), and death (75 to 85% of the animals) (Table 2). These results are compatible with the usual data reported in our model (4, 6, 25, 26). In these groups, weight loss started in some animals as soon as 3 days after challenge, profuse watery diarrhea usually first appeared 4 days after challenge, and the first mortalities took place 1 or 2 days after the onset of diarrhea. In some rabbits, the symptoms were delayed, perhaps as a result of multiple reinfections by the challenge strain excreted by ill animals. In groups vaccinated with Formalin-killed preparations (Table 2), the homologous vaccine (group KB10) provided complete protection against diarrhea and mortality, with only transient growth retardation in one animal. Vaccine strains C127 and C6 provided only partial or poor protection; reduction of weight loss, diarrhea, and mortality were significant in group KC127 compared with the control group, but protection was only about 50 to 60%, while only mortality was significantly reduced in group KC6, with a low percentage of protection. In groups vaccinated with live preparations, both vaccinia strains yielded interesting results; only one animal vaccinated with C127 experienced symptoms and died of diarrhea, while neither diarrhea nor mortality was recorded in group LC6. In the latter, however, growth retardation episodes were noted in half of the animals (Table 2) and were all related to colonization by the vaccinia strains, as they occurred before challenge (i.e., days 3 to 7). Although these growth retardation periods were short and the weight loss ranged from only 15 to 40 g per animal, they significantly altered the mean daily weight gain of the group (Table 2).

**Enumeration of fecal or cecal *E. coli.* In all dead animals from control as well as vaccinated groups, necropsy findings (distended cecum, with fluid content; hemorrhagic "paintbrush" lesions of cecal mucosa in half of the cases) were compatible with O103 colibacillosis. Fecal contents sampled after death contained more than 10⁹ CFU of *E. coli* per ml (range, 1.3 × 10⁷ to 2.0 × 10⁹). All the strains subcultured and tested for serogroup and biotype (six clones per sample) were found to be identical to challenge strain B10. Usually, no significant coccidial coinfections were detected in the samples, except for two animals in the control group (2 × 10⁴ oocysts per ml), one in group KC127 (3 × 10⁴ oocysts per ml), and one in group KC6 (1.2 × 10⁴ oocysts per ml). Taken together, the results agree with challenge-induced O103 colibacillosis as the cause of disease and death of the animals. The kinetics of fecal *E. coli* colonization in control and vaccinated animals are shown in Fig. 1 and 2. In both control groups, the challenge strain quickly colonized the

**TABLE 2. Clinical manifestations observed in animals vaccinated with Formalin-killed strains (strains B10 [KB10], C127 [KC127], and C6 [KC6] and live strains (strains C127 [LC127] and C6 [LC6]) and control animals after challenge with pathogenic O103 *E. coli* B10**

<table>
<thead>
<tr>
<th>Vaccine and group</th>
<th>No. of animals affected with the following/no. of animals in the group:</th>
<th>Mean daily wt gain, in g of survivors (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt loss</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Formalin killed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>25/26</td>
<td>24/26</td>
</tr>
<tr>
<td>KB10</td>
<td>1/13</td>
<td>0/13</td>
</tr>
<tr>
<td>KC127</td>
<td>10/24^d</td>
<td>9/24^d</td>
</tr>
<tr>
<td>KC6</td>
<td>18/25^d</td>
<td>18/25^d</td>
</tr>
<tr>
<td>Live</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None^a</td>
<td>21/24</td>
<td>19/24</td>
</tr>
<tr>
<td>LC127</td>
<td>1/12^c</td>
<td>1/12^c</td>
</tr>
<tr>
<td>LC6</td>
<td>6/12^d</td>
<td>0/12^d</td>
</tr>
</tbody>
</table>

^a See Table 1 for strain characteristics. Levels of significance are reported relative to the control group.

^b V to C, between vaccine and challenge; C to S, from challenge to slaughter.

^c P < 0.001 (Chi-square test).

^d Not significant.

^e P < 0.001 (Student’s t test).

^f P < 0.05 (Student’s t test).

^g P < 0.05 (Chi-square test).

^h Sum of two separate control groups.

^i P < 0.05 (Student’s t test).
intestinal tract to yield fecal outputs of more than $10^6$ CFU 5 days after infection. At this time, all the strains recovered in the feces were phenotypically identical to the challenge strain. This colonization persisted until the end of the experiment; a decrease in CFU counts and a reappearance of the strains of the normal flora did not occur until days 29 to 32. In groups KB10, KC127, and KC6, B10 colonization was delayed and did not completely exclude the normal E. coli flora (except in KC6 at day 21; Fig. 1). In KB10, the total E. coli and challenge strain outputs were significantly lower than those in the control, and in all three vaccinated groups, the proportions of the recovered challenge strain correlated quite well with the intensity of clinical signs. In groups LC6 and LC127 (Fig. 2), the situation was quite different. Strain C127 colonized the intestinal environment 5 days after administration at a mean level of only $6.18 \pm 0.55 \log_{10}$ CFU/g of feces, although it had been shown to adhere in vitro to intestinal villi of 6-week-old rabbits (26). At this time, all the E. coli strains recovered had the C127 phenotype. In this group, strain B10 was reisolated on days 5 and 12 after challenge (i.e., days 12 and 19 after vaccination, respectively; Fig. 2), but the extent of its colonization was significantly lower than that in the control group. Strain C6, on the contrary, colonized heavily the digestive tract of rabbits of group LC6 (mean $\log_{10}$ CFU/g, $7.93 \pm 0.61$ at day 5, $8.64 \pm 0.36$ at day 12, and $7.51 \pm 1.32$ at day 19). Furthermore, it completely excluded strain B10, which could not be recovered at any time after challenge (Fig. 2).

**Fecal anti-LPS IgA antibodies.** Fecal IgA responses against O103 or O128 LPS are shown in Fig. 3 and 4. Different kinetics were obtained in rabbits vaccinated with killed or live strains. In group KB10 or KC127 (Fig. 3), responses against LPS O103 were not detected, in contrast to previous results (26), in which a small, although significant, rise in the levels of anti-O103 LPS IgA antibodies had been detected in the feces of vaccinated animals compared with control animals. It must be stressed that in situations in which vaccine and challenge strains share the same LPS, the vaccine response may be difficult to detect because of the complexion of specific IgA with the challenge bacteria. Anti-O103 LPS IgA antibodies were only detected in fecal samples of group KC6 and the controls group 21 days after challenge and could therefore be explained by the heavier colonization of challenge strain B10. When tested with O128 LPS, the same samples yielded the expected results, with a slight, although significant, rise in anti-O128 LPS IgA antibodies in group KC6 only.

In group LC127 or LC6 (Fig. 4A and B), the levels of IgA directed against the LPS of vaccine strains increased regularly after vaccination, and significant levels could be detected 12 days after vaccine administration. These results show the greater ability of live vaccines to stimulate local immune responses. In group LC6 (Fig. 4B), no anti-O103 LPS IgA antibodies were detected any time after challenge, a result that may be correlated with the “resistance to colonization” induced by the vaccine strain against the challenge one. Control groups yielded the expected results, with a postchallenge rise in anti-O103 LPS IgA antibody levels at the end of the observation period. As part of the protection mechanisms, the antiadhesin antibody response...
FIG. 2. Kinetics of fecal E. coli colonization in animals vaccinated with live C127 (LC127) or C6 (LC6) and in corresponding control (nonvaccinated) animals. All the rabbits were challenged with \(2 \times 10^5\) of B10 CFU (black arrows) 7 days after the oral administration of the vaccine strain (white arrows). The ordinate indicates the mean \(\log_{10}\) CFU of E. coli per gram or milliliter of feces. Hatched, grey, and white bars indicate, respectively, the proportions of CFU of the vaccine strain (C127:0103, rhamnose positive; C6: 0128, rhamnose positive), B10 challenge strain (0103, rhamnose negative), and irrelevant E. coli from the normal flora (non-0103, non-0128, rhamnose positive) identified in the total E. coli population. *P values above bars indicate the significance of the mean total E. coli count compared with that of the control group (Student's t test). **P values in the grey bars indicate the significance of the challenge E. coli B10 count compared with that of the control group (Student's t test). NS, not significant.

might be involved, too, but we were unable to detect any fecal IgA directed against a B10 surface extract in which adhesin was the prominent component in the animals of group LC6 (data not shown).

Colicinlike activity of O128 strains against pathogenic O103 E. coli. To explain the drastic ecological effect observed in vivo when strain C6 was administered before challenge strain B10, we tested vaccine strains C127 and C6, together with two other strains with analogous properties (C124 and C104; Table 1), for their in vitro ability to inhibit the growth of several highly pathogenic O103 strains (B10, C55, C70, C148, and C199; Table 1). Both O128 strains C6 and C104 were able to inhibit the growth of all the O103 strains tested, while the nonpathogenic O103 strains were not (Fig. 5). This result suggests a specific colicinlike activity of O128 strains against pathogenic O103 strains that might have been involved in the ecological barrier effect observed in vivo when strain C6 was administered before strain B10 (group LC6).

**DISCUSSION**

E. coli is a bacterial species frequently found in the intestinal flora of rabbits (17, 23). These resident strains are nonpathogenic but are thought to proliferate in abnormal situations, such as zootechnic errors, stress, or adverse external conditions (high or low ambient temperature) (39), or during coinfections with parasites (Eimeria spp.) (22) or perhaps viruses (rotavirus) (34). When given per os to healthy rabbits, these strains do not cause clinical signs. On the contrary, since the early 1980s, typical strains with high experimental pathogenicity have been recovered in weaned rabbits experiencing diarrhea in French or Belgian fattening units (2, 21, 33, 36). In France, epidemiological data and the fulfillment of Koch's postulates have led to the conclusion that the strains involved in that specific pathology belong mainly to serovar O103:H2– or less frequently to serovar O26:H11– and to rhamnose-negative biotypes (5). Control
VACCINATION OF RABBITS AGAINST EPEC-LIKE E. COLI O103

FIG. 4. Kinetics of fecal anti-O103 LPS IgA antibodies or anti-O128 LPS IgA antibodies in animals receiving live vaccine (groups LC127 [A] and LC6 [B]) and control (nonvaccinated) ones. Data are the mean ± standard error of the mean. * There was only one animal remaining in this control group. Significance was determined by Student’s t test. C., control; ELISA, enzyme-linked immunosorbent assay; SOD, specific OD.

FIG. 5. In vitro growth inhibition of highly pathogenic O103 strains (streaked C55, B10, C70, C148, and C199) by 32K adhesin-negative, nonpathogenic O128 strains (spotted C6 and C104). Spotted strains C127 and C124 (32K adhesin-negative, nonpathogenic O103 strains) did not have this property.

of the disease by antibiotics is unsatisfactory and does not permit eradication of the strains from an infected farm (8, 32). Vaccination experiments have led us to conclude that oral Formalin-killed vaccines may be of interest in preventing and eradicating O103 infections in weaned rabbits (6, 7, 26). However, the use of oral inactivated vaccines is tedious, as it requires repeated administration of high doses of antigen, a condition which is technically and economically difficult in field practice. Therefore, it would be interesting to use live attenuated vaccine strains, which could be given once at a low dose, could multiply in vivo, and would better protect the animals via a strong stimulation of the gut-associated lymphoid tissue (24, 29). In an attempt to test this hypothesis, we used live nonpathogenic E. coli strains that shared the LPS or adhesin of highly pathogenic O103 strains to protect weaned rabbits against challenge. These strains were used as Formalin-killed vaccines, too, in a comparison with the homologous strain in a vaccination schedule which had been shown to protect the animals (6, 26).

When used after inactivation, strain B10 completely protected the animals against a homologous challenge, with significantly hampered colonization by the challenge dose, resulting in a low postchallenge anti-O103 LPS fecal IgA response. These results are in agreement with our previous ones (6, 26). Strain C6 (O128, rhamnose positive, 32K adhesin positive) resulted in little protection, with a delay in colonization by challenge strain B10 compared with that in the control, and slight but significant protection against mortality. Strain C127 (O103, rhamnose positive, 32K adhesin negative) induced partial protection against challenge strain B10, with a delay in colonization after challenge, and a partial but significant reduction in weight loss, diarrhea, and mortality. With both O103 strains B10 and C127, anti-O103 LPS fecal IgA antibodies were not detected after vaccination (contrary to the detection of anti-O128 LPS fecal IgA antibodies in the group vaccinated with C6), but the postchallenge rise was detected mainly in the group vaccinated with C6 and the control group. The following conclusions may be drawn from these results. (i) Local anti-LPS IgA antibodies are difficult to detect when the corresponding antigen is present in the digestive tract of animals (i.e., immunizing antigen at a high dose, then colonizing live strain harboring the antigen). This conclusion confirms our earlier findings (6, 26). There may be several reasons for this conclusion. First, young weaned rabbits may be ontogenetically unable to respond to an LPS stimulus as soon as 30 days of age. This explanation seems unsatisfactory, however, as local anti-O128 LPS IgA antibodies were detected in group KC6. Second, the overwhelming quantity of the colonizing challenge strain may fix all the specific IgA present as an immune complex and eliminate it, making it undetectable in an enzyme-linked immunosorbent assay. We showed that an inactivated vaccine was able to induce detectable anti-O103 LPS IgA antibodies in feces and milk of does after vaccination and without a subsequent challenge (25). Cantey et al. (10), using the RDEC-1 model, also stressed the influence of diarrhea on the difficulties in detecting local IgA responses in rabbits. However, this explanation does not agree with our results, as none of the rabbits in group KB10 experienced diarrhea. (ii) A postchallenge response is detectable after heavy colonization by the challenge strain, i.e., in animals surviving challenge in the least protected groups, and when the colonization begins to decrease. (iii) LPS is an important protection-inducing antigen, as judged by the partial protection afforded by strain C127, but it is not the only one, and the 32K adhesin may play a role, even in inactivated vaccine formulations.
At the moment, live bacterial strains that may be used in oral vaccinations against enteric bacteria, such as *Salmonella* or *Shigella* spp., are being extensively studied. These include *Salmonella typhi* gallin mutant Ty21a, which is used for vaccination against typhoid fever (16, 20), *Salmonella* mutants with deletions in *aroA* (18, 41), *cya* and *crp* (11), porin genes (12), or *phoP* (15), or *ics-icu* mutants of *Shigella* spp. (14). Such strains are also candidates as vectors for foreign antigens in diseases in which local enteric stimulation seems to be needed (35, 37, 38). Live attenuated bacterial vaccines have many advantages: low doses, self in vivo multiplication, sometimes natural targeting to Peyer’s patches, induction of strong local immune responses at initial and remote mucosal surfaces via GALT stimulation, and generalized mucosal responses (24, 29). In our model, live vaccines are of interest in enabling practical and economical improvement of vaccination schedules in field practice. Strains determined experimentally to be nonpathogenic were used here as an approach to vaccination with live material. Both of the strains used resulted in significant protection against challenge. O103 strain C127 colonized the digestive tract of the animals quite well and elicited an anti-O103 LPS IgA response which may have been involved in protection. The kinetics of the antibody response were compatible with the results of Cantey et al. (10) who, using the enteropathogenic *E. coli*-like RDEC-1 model, observed a local anti-LPS response in digestive contents as well as in remote mucosal sites (bronchi) and in serum after infection with O15:H− strain RDEC-1 (9). The similar kinetics of the anti-O128 LPS IgA response observed in group LC6 led to the conclusion that these antibodies were induced by vaccination and not by challenge. In group LC127, protection seemed to be fairly well correlated with the anti-O103 LPS IgA response, which precluded heavy colonization by the challenge strain, and this result confirms the importance of LPS as the major protection-inducing antigen. In rabbits vaccinated with 32K adhesin-positive strain C6, protection against challenge was complete. This result may have been directly due to the ecological competition between both strains, which harbor the same adhesin, with strain C6, which bears colicin-like activity against O103 strains, being more competitive. To our knowledge, direct evidence of the role of colicins in colonization of the digestive tract and/or competitive exclusion by producing strains is lacking. Smith and Huggins (40) demonstrated in a study of human volunteers that colicin V-producing *E. coli* strains showed enhanced intestinal survival compared with nonproducing strains. As far as colicin V (the oldest colicin known) is concerned, a recent review stressed the lack of critical experiments on its role in vivo (43). Nevertheless, strain C6, which was supposed to be nonpathogenic, according to previous experiments done with a small number of animals (5), was shown here to induce significant weight losses in the vaccinated animals during the colonization phase. Because of its weight loss-inducing capacity, this strain cannot be used in field trials, but it may be of interest to test it as a therapeutic agent in rabbits experiencing symptoms of O103 colibacillosis and to determine whether it may act in a competitive way on a preexisting O103 flora, too. The apparent competitive exclusion of O103 strain B10 by strain C6 provides an interesting model to test the potential role of colicins in such a phenomenon.

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