Safety and Immunogenicity of *Salmonella enterica* Serovar Typhimurium *ltaB* in Mice

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Abstract The safety and immunogenicity of an attenuated recombinant *Salmonella* vaccine strain, *Salmonella enterica* serovar Typhimurium *ltaB*, was assessed. This vaccine strain could survive in low pH condition, and its ability of intracellular survival did not differ from that of *S. enterica* serovar Typhimurium UK1, which is the wild-type of the vaccine strain. The mortality of the mice orally administered with the vaccine strain was 50% at the dose of 10^6 CFU. All mice administered with 10^6 or 10^7 CFU of the vaccine strain survived for 3 days postinoculation (pi). However, all mice administered with more than 10^6 CFU of the vaccine strain died within 3 days pi. To examine the protective effect of the vaccine strain, mice were orally immunized with 10^6 and 10^7 CFU of the bacteria. Control mice were given with 0.5 ml of phosphate buffered saline (PBS). After 8 days, the mice were challenged with 10^6 CFU of *S. enterica* serovar Typhimurium UK1, and mortality was examined for 5 days. The survival rates of the mice immunized with 10^6 and 10^7 CFU of the vaccine strain were 60% and 80%, respectively, whereas all control mice died within 2 days after challenging. To investigate the immunogenicity of *S. enterica* serovar Typhimurium *ltaB*, mice were orally immunized with 10^6 or 10^7 CFU ml of the vaccine strain. Five mice of each group were sacrificed at 5 and 12 days after immunization, and results showed that immunization of the vaccine strain led to increases of IgG1, IgG2, and IgM titers against *S. enterica* serovar Typhimurium UK1 in mouse sera, cytokine expressions such as IL-2, IL-4, IL-6, and IL-10 in spleen, and the lymphocyte proliferation response to mitogens (concanaavalin A or LPS) stimulation.

Key words: Attenuated recombinant *Salmonella* vaccine, *S. enterica* serovar Typhimurium *ltaB*, safety, immunogenicity

According to the World Health Organization (WHO), about 16 million cases of typhoid fever have been reported annually, and close to 600,000 deaths occur in areas of endemicity in Africa and Asia [11, 16, 17]. Chloramphenicol and ampicillin have been effectively used for many years, but treatment and control of typhoid fever have become increasingly difficult, because strains of *S. enterica* serovar Typhi resistant to these antibiotics have emerged worldwide [19].

Live oral typhoid vaccine Ty21a is one of the safest and best tolerated vaccines of all licensed vaccines [3, 24]. However, the modest immunogenicity of this vaccine, which requires three or four spaced doses (with an interval of 48 h between doses) to confer the credible protection, constitutes an important and practical shortcoming [3, 6, 8, 9, 13, 21]. These shortcomings stimulated efforts to identify alternative attenuated *S. enterica* serovar Typhi and *S. enterica* serovar Typhimurium strains that are well tolerated as Ty21a but much more immunogenic, so that they can be administered as single-dose live oral vaccines. *S. enterica* serovar Typhimurium is a Gram-negative facultative intracellular bacterium that causes systemic infection in mice, similar to human typhoid fever [1, 16]. Therefore, *S. enterica* serovar Typhimurium has been considered as a suitable strain to study human typhoid fever and as a candidate vector in vaccine design [23]. Recently, attenuated *Salmonella* spp. gained attention, because they can be administered mucosally and stimulate humoral and cell-mediated immune

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response [16, 18–20, 22]. A variety of attenuated *Salmonella*
strains such as CDV 908, CDV 906, CDV 906-htrA, phoP/
phoQ, and rpoSR mutants have been used as live vaccines
to induce mucosal as well as systemic immunity against
either carrier itself or heterologous antigen expressed [6, 8,
13, 22, 24]. Recombinant *Salmonella* vaccines have also
been developed as multivalent vaccines to deliver recombinant
antigens originating from viruses, bacteria, and parasites
[24]. Also, live attenuated *Salmonella* strains that express a
foreign antigen can be administered via the oral route and
can induce strong mucosal and systemic immune response,
to the foreign antigen, conferring protective immunity against
numerous pathogens in several animal models. Despite
encouraging preclinical results, however, a few clinical trials
with live recombinant *Salmonella* strains showed weak to
undetectable human immune response to the foreign antigens,
indicating the need for further optimization [19]. We
developed an attenuated recombinant *Salmonella* vaccine
strain, *S. enterica* serovar Typhimurium *llaB*. In the present
study, the safety and immunogenicity of this vaccine
candidate in mice were evaluated.

**Materials and Methods**

**Mice**

Four-week-old specific pathogen-free BALB/c male mice
(Orient, Seoul, Korea) were used in this study. They were
housed in individual cages at 22±2°C under a 14/10 h light/
dark cycle. Food and water were provided *ad libitum*. All
animal experiments were performed in accordance with
the laboratory animal guidelines of Seoul National University.

**Bacterial Strain**

Bacteria were cultured at semi-aerobic conditions in 3 ml
of medium in 13×100 mm test tubes with shaking of
240 rpm at 37°C. The bacterial strain used in this study
was *S. enterica* serovar Typhimurium *llaB::MudJ*. Acid-
sensitive mutant, *S. enterica* serovar Typhimurium *
llaB*, was isolated using P22-mediated MudJ (Km, lacZ) transduction of a wild-type *S. enterica* serovar Typhimurium UK1, as
described by Holly and Foster [7]. About 20,000 transductants
were patched onto LB plates with kanamycin (50 μg/ml)
and grown overnight at 37°C. Grown patches were replicated
to LB plates containing dinitrophenol (200 μM) and incubated
at 37°C. Survivors were rescued after 5 h by replicating
onto LB plates [4]. Expected mutants that did not survive
under this treatment were restricted from the original master
plates, and named *S. enterica* serovar Typhimurium *llaB*.

**Acid Tolerance Response**

To examine the pH tolerance of *S. enterica* serovar
Typhimurium *llaB* and *S. enterica* serovar Typhimurium
UK1, the pH of LB broth was adjusted to 3.0, 5.0, 6.0, and
7.2 (control) by addition of NaOH. Two-hundred μl of
overnight-cultured *S. enterica* serovar Typhimurium *llaB*
and *S. enterica* serovar Typhimurium UK1 were added to
each tube containing 10 ml each of pH-adjusted LB broth
and incubated at 37°C. OD at 600 nm was measured at 0,
0.5, 1, 2, 4, 5, and 6 h post-incubation at 37°C with shaking
of 220 rpm. At that time, the viable bacteria were counted
by plating onto LB agar. This process was performed
twice.

**Invasion and Intracellular Survival Assay**

HT-29 human colon adenocarcinoma cells (Korean Cell
Line Bank: KCLB, Seoul, Korea) were cultured and maintained
in RPMI-1640 medium supplemented with 10% (v/v) fetal
bovine serum (Gibco, Grand Island, NY, U.S.A.) and 1 mM
penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO,
U.S.A.). The cells were grown at 37°C in a 5% CO2
atmosphere. Prior to invasion assays, cells were passaged
several times in the absence of antibiotic. One ml of
RPMI-1640 medium containing 4×106 cells was added into
each well of a 24-well tissue culture plate and incubated
overnight. Bacteria were incubated overnight in RPMI-1640
medium without antibiotics, and the number of bacteria
was estimated by measuring the OD of the culture at 600 nm.
Approximately 107 CFU of the bacteria were added to HT-
29 cells and incubated for 4, 24, 48, and 72 h. Cells were
subsequently washed three times with phosphate buffered
saline (PBS; pH 7.2), and the growth of extracellular bacteria
was inhibited by replacing the medium with RPMI medium
containing 50 μg of ampicillin per ml. Cells were further
incubated for 1 h, washed three times with PBS, and lysed
by addition of 1 ml of 1% Triton X-100 (Sigma, U.S.A.) in
PBS for 30 min at 37°C. The plates were shaken vigorously
for 5 min to ensure lysis. The lysates were serially diluted
ten-fold in PBS and plated onto LB agar.

**Virulence and Efficacy of *S. enterica* Serovar Typhimurium *llaB***

To examine the virulence of *S. enterica* serovar Typhimurium
*llaB*, overnight-incubated *S. enterica* serovar Typhimurium
*llaB* were diluted with PBS to make concentrations of 106,
105, 104, and 103 CFU/0.5 ml. Ten mice of each group were
orally administered with *S. enterica* serovar Typhimurium
*llaB*, and mortality was then examined for three days. To
examine the efficacy of *S. enterica* serovar Typhimurium
*llaB*, five mice of each group were orally administered with
106 and 105 CFU/0.5 ml of *S. enterica* serovar Typhimurium
*llaB*. At 8 days after immunization, mice were orally challenged
with 106 CFU/0.5 ml of *S. enterica* serovar Typhimurium
UK1, and mortality was examined for five days.

**Immunogenicity of *S. enterica* Serovar Typhimurium *llaB***

Mice of each group were orally immunized with 106 and
105 CFU/0.5 ml of *S. enterica* serovar Typhimurium *llaB*
grown, respectively, while the control mice were administered with 0.5 ml of PBS. At 5 and 12 days after immunization, all mice were anesthetized with ether, and blood samples were collected by a cardiac puncture using a heparin-treated syringe. The mice were then necropsied, and spleens were sampled.

**Proliferation Assay of T- and B-Cells in Spleen.** The spleens were removed aseptically from mice and placed individually into Petri dishes containing 3 ml of complete RPMI-1640 medium. As previously described [2, 12], single-cell suspensions were prepared by chopping the spleens into small pieces with sterile scissors and then forcing the spleen tissue up and down through a 3 ml syringe. The suspension was transferred to a 15 ml conical tube containing 3 ml of complete RPMI-1640 and centrifuged at 1,700 rpm for 10 min. The cell pellet was resuspended in ACK lysis buffer (Tris-NH4Cl) and incubated for 15 min with occasional mixing to lyse erythrocytes. After washing twice in complete RPMI-1640, the suspensions were adjusted to a final concentration of 2 × 10^6 cells/ml in complete RPMI-1640. Proliferation responses of spleen cells to mitogens were determined using a commercial cell proliferation ELISA kit (Roche Diagnostics, Mannheim, Germany). Briefly, 10^5 cells in 50 μl of complete RPMI-1640 medium were added to the wells of a 96-well tissue culture plate and cultured in the presence or absence of T- and B-cell mitogens. Fifty μl of concanavalin A (Con A, 2.5 μg/ml) and lipopolysaccharide (LPS, 5 μg/ml) were added to the wells. Control wells received 50 μl of complete medium. The cells were cultured for 72 h at 37°C in a humidified incubator supplemented with 5% CO₂. Cell proliferation during the final 18 h of culture was determined by measuring the incorporation of 5-bromo-2-deoxyuridine (BrdU) using peroxidase-conjugated anti-BrdU antibodies and a peroxidase substrate system. The absorbance of each well was read at 450 nm using an ELISA reader (Sunrise, TECAN Austria GmbH, Salzburg, Austria).

**Estimation of Antibody Responses.** An ELISA was used to examine the antibody level to *S. enterica* serovar Typhimurium UK1. *S. enterica* serovar Typhimurium UK1 was cultured in tryptic soy broth and collected. The bacteria were washed three times with sterilized PBS and broken with a sonicator. Twenty-five μg of total bacterial protein were loaded into each well of a 96-well ELISA plate and incubated overnight at 4°C. After washing three times with PBS, the plate was blocked with 1% bovine serum albumin at 4°C for 2 h and incubated with sera (1:50 diluents) of mice at room temperature (RT) for 2 h. Then, it was washed three times with 0.05% Tween-PBS (T-PBS) and incubated with peroxidase-conjugated anti-mouse IgG1, IgG2a, and IgA antibodies (Zymed, San Francisco, CA, U.S.A.) at room temperature (RT) for 2 h. Finally, it was washed three times with T-PBS, visualized with o-phenylenediamine dihydrochloride (Sigma, U.S.A.), and absorbance was read at 450 nm.

**Semi-Quantitative RT-PCR for Cytokines.** A portion of spleen samples was added to 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.), and was electrically homogenized (Ultraturrax T8, IKA, Heidelberg, Germany) in 5-ml round-bottom glass tubes in 500 μl of Trizol on ice. Each homogenate was transferred to a 1.5-ml Eppendorf tube, vortex-mixed, and incubated at RT for 5 min. Two-hundred μl of chloroform were added, and the tubes were vortex-mixed for 15 s and placed on ice for 10 min. Phases were separated by centrifugation at 12,000 rpm for 20 min in a refrigerated microcentrifuge. The upper aqueous phase was transferred to a new 1.5 ml tube, and RNA was precipitated by the addition of 0.5 ml of isopropanol. The tubes were allowed to stand at −20°C for at least 12 h. The precipitate was pelleted by centrifugation at 12,000 rpm for 15 min at 4°C, and the pellets were washed with 1 ml of 70% ethanol. After removal of the supernatant, the pellets were dried at RT for 20 min before resuspension in 100 μl of diethyl pyrocarbonate-treated water. The RNA concentration was determined by absorbance at 260 nm. cDNA was reverse transcribed from 1 μg of total RNA using a GeneAmp RNA PCR Core kit (Applied Biosystems, Foster City, CA, U.S.A.). PCR was performed using i-Taq DNA polymerase (iNTRON, Sungnam, Kyungki-

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Sequences (5'-3')</th>
<th>Annealing temp (°C)</th>
<th>Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>F: CCCCATGATGCTCACGTATTA*&lt;br&gt;R: TTCCAGGCACCTGGAAGTGT</td>
<td>54</td>
<td>73</td>
</tr>
<tr>
<td>IL-4</td>
<td>F: CAGAGCTATTTGATGGTCTCA*&lt;br&gt;R: TGTTGGCTCAGTACTAGGATG</td>
<td>54</td>
<td>446</td>
</tr>
<tr>
<td>IL-6</td>
<td>F: CATGTTCTCCTGGGAAATCGTG*&lt;br&gt;R: AACGTAGTATGCTTGGCATACGCAC</td>
<td>52</td>
<td>492</td>
</tr>
<tr>
<td>IL-10</td>
<td>F: AGCTGGACACACATATGCTAACCC&lt;br&gt;R: TTTTCCAGGAGTTGTTTCCGTTAG</td>
<td>52</td>
<td>461</td>
</tr>
<tr>
<td>β-actin</td>
<td>F: ATGGATGAGCATATGCT&lt;br&gt;R: ATGAGGTAGTTCGATCAGGT</td>
<td>56</td>
<td>569</td>
</tr>
</tbody>
</table>

*Designed by the primer selection program of BCM Search Launcher (Baylor College of Medicine, Human Genome Sequence Center, Houston, TX, U.S.A.) and used for sequencing of the 16S rRNA gene.

*From reference 9.
Do, Korea) and specific primer sets for cytokines. The sequences of the primers for cytokines, annealing temperature, and sizes of PCR products are shown in Table 1. PCR conditions were as follows: 3 min of denaturation at 95°C, followed by 35 cycles consisting of 45 sec of denaturation at 94°C, 45 sec of annealing at 52–54°C, and 1 min of extension at 72°C, and 5 min of final extension at 72°C after amplification. The PCR products were resolved on 1.5% agarose gel with ethidium bromide and analyzed using a model Gel-Doc 2000-Quantity one Discovery Series™ (BIO-RAD Laboratories, Inc., Hercules, CA, U.S.A.) and Kodak Digital Science 1D-Scientific Imaging System software (Kodak, New Haven, CT, U.S.A.). The scanned images were digitized and quantified using Kodak Digital Science 1D analysis software, and the values for each cytokine were compared to those of β-actin. Each sample in all experiments was analyzed in duplicate.

**Statistical Analysis**
Significant differences between the experimental and control groups were determined using Duncan’s Multiple Range Test (SAS ver. 8.1, SAS Institute Inc., Cary, NC, U.S.A.). Values of p<0.05 were considered significant.

**RESULTS**

**Acid Tolerance and Intracellular Survival Assay**
The pH tolerance of *S. enterica* serovar Typhimurium UK1 and *S. enterica* serovar Typhimurium *llaB* were evaluated (Fig. 1). Both bacteria incubated in pH 7.2 or 6.0-adjusted medium grew continuously; however, the growth curve of *S. enterica* serovar Typhimurium UK1 incubated in pH 3.0 or 5.0-adjusted medium decreased after 30 min. On the other hand, *S. enterica* serovar Typhimurium *llaB* incubated in pH 3.0 or 5.0-adjusted medium showed growth pattern similar to that incubated in pH 7.2 or 6.0-adjusted medium. In the intracellular survival assay using HT-29 cell, the number of both intracellular bacteria decreased with time. *S. enterica* serovar Typhimurium *llaB* strain showed a significantly lower intracellular survival rate than that of *S. enterica* serovar Typhimurium UK1 at 2, 48, and 72 h (Fig. 2).

**Virulence and Efficacy of *S. enterica* Serovar Typhimurium *llaB***
The virulence of *S. enterica* serovar Typhimurium UK1 and *S. enterica* serovar Typhimurium *llaB* in mice was evaluated. All the mice administered with 10⁷ CFU or more of *S. enterica* serovar Typhimurium UK1 died. All the mice administered with 10⁷ CFU of *S. enterica* serovar Typhimurium *llaB* died within 3 days p.i.; however, the mice administered with 10⁷ CFU of the bacteria showed 50% mortality (Table 2). None of the mice administered with 10⁶ or 10⁸ CFU of *S. enterica* serovar Typhimurium *llaB* died (Table 2). Based on these results, 10⁷ and 10⁸ CFU of

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**Fig. 1.** Acid tolerance response of *S. enterica* serovar Typhimurium UK1 (A) and *S. enterica* serovar Typhimurium *llaB* (B). Results are expressed as mean±SD. Each symbol indicates pH 3.0 (■), 5.0 (▲), 6.0 (▼), and 7.3 (●).

**Fig. 2.** Intracellular survival of *S. enterica* serovar Typhimurium UK1 (■) and *S. enterica* serovar Typhimurium *llaB* (▲) in intestinal epithelial cell line HT-29. Results are expressed as mean±SD.
Table 2. The virulence of *S. enterica* serovar Typhimurium UK1 and *S. enterica* serovar Typhimurium *llaB*.

<table>
<thead>
<tr>
<th>Inocula dose (CFU)</th>
<th><em>S. enterica</em> serovar Typhimurium UK1</th>
<th><em>S. enterica</em> serovar Typhimurium <em>llaB</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁸</td>
<td>0/10</td>
<td>10/10</td>
</tr>
<tr>
<td>10⁷</td>
<td>0/10</td>
<td>10/10</td>
</tr>
<tr>
<td>10⁶</td>
<td>ND¹</td>
<td>10/10</td>
</tr>
<tr>
<td>10⁵</td>
<td>0/10</td>
<td>5/10</td>
</tr>
<tr>
<td>10⁴</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

¹Mortality was examined for three days.

*Not determined.

*S. enterica* serovar Typhimurium *llaB* were determined to be the immunizing dose for the efficacy test. When challenged with 10⁷ CFU of *S. enterica* serovar Typhimurium UK1, the survival rate of the mice immunized with 10⁷ or 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* were 60 and 80%, respectively (Fig. 3). However, all control mice administered with PBS instead of *S. enterica* serovar Typhimurium *llaB* died within 2 days after challenging with *S. enterica* serovar Typhimurium UK1.

**Proliferation Assay**

The proliferative responses of spleen cells isolated from the mice administered with 10⁷ and 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* to stimulation with ConA were significantly higher than those from the control mice at 5 and 12 days after immunization (p<0.05, Fig. 4). The proliferation of B-cells in response to stimulation with LPS in the spleen of the mice administered with 10⁷ or 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* was significantly higher than in that of control mice at 5 days after immunization.

![Fig. 3. The survival rate of mice after challenging with *S. enterica* serovar Typhimurium UK1.](image)

Mice of each group were orally immunized with 10⁷ (–△–) or 10⁶ CFU (–□–) of *S. enterica* serovar Typhimurium *llaB*. Control mice (–▽–) received 0.5 ml of PBS.

![Fig. 4. Proliferation of spleen cell in response to stimulation of Con A or LPS at 5 and 12 days after immunization.](image)

Mice of each group were orally immunized with 10⁷ (the bar with the oblique line) or 10⁶ (the dark bar) CFU of *S. enterica* serovar Typhimurium *llaB*. Control mice (the white bar) received 0.5 ml of PBS. Results are expressed as mean±SD. *Means with the same letter are not significantly different (p< 0.05).

However, the B-cells proliferation was significantly higher in the spleen of 10⁷ CFU-immunized mice, but not 10⁶ CFU-immunized mice, than in that of control mice at 12 days after immunization (p<0.05, Fig. 4).

**Estimation of Antibody Responses**

Five days after immunization, titers of all tested immunoglobulins such as IgG₁, IgG₂, IgA, and IgM were significantly higher in sera of the mice administered with 10⁷ and 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* than in those of control mice, except that of IgG₂ in sera of the mice with 10⁶ CFU of the bacteria (p<0.05, Table 3). At 12 days after immunization, sera IgG₁, IgG₂, IgA, and IgM titers of all the mice administered with 10⁷ and 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* were significantly higher than those of control mice (p<0.05, Table 3). Except for sera IgM, the titers of all immunoglobulin types at 12 days after immunization were higher than those at 5 days after immunization. There was no significant difference in antibody titer between the groups administered with 10⁷ and 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* (p<0.05).

**Semi-Quantitative RT-PCR for Cytokines**

mRNA expressions of IL-2, IL-4, IL-6, and IL-10 were significantly higher in the spleens of the mice administered with *S. enterica* serovar Typhimurium *llaB* than in those of control mice. The expression of all cytokines in the spleen was dose dependent; however, they did not show any significant difference between the groups administered with 10⁷ and 10⁶ CFU of *S. enterica* serovar Typhimurium *llaB* (p<0.05, Table 4). The expression levels of all cytokines...
Table 3. ELISA for detection of the levels of IgG1, IgG2a, IgA, and IgM from sera of mice immunized with 10^8 or 10^9 CFU of S. enterica serovar Typhimurium llaB at 5 and 12 days after immunization.

<table>
<thead>
<tr>
<th></th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgA</th>
<th>IgM</th>
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<tr>
<td>5 days</td>
<td></td>
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<tr>
<td>Control</td>
<td>0.087±0.008b</td>
<td>0.085±0.003b</td>
<td>0.104±0.005b</td>
<td>0.118±0.003b</td>
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<tr>
<td>llaB 10^8 CFU</td>
<td>0.169±0.023a</td>
<td>0.092±0.009ab</td>
<td>0.142±0.048a</td>
<td>0.348±0.021a</td>
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<tr>
<td>llaB 10^9 CFU</td>
<td>0.172±0.031b</td>
<td>0.102±0.015a</td>
<td>0.139±0.037a</td>
<td>0.376±0.031b</td>
</tr>
<tr>
<td>12 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.125±0.013b</td>
<td>0.072±0.008b</td>
<td>0.115±0.006a</td>
<td>0.160±0.021b</td>
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<tr>
<td>llaB 10^8 CFU</td>
<td>0.266±0.069a</td>
<td>0.158±0.032b</td>
<td>0.221±0.041a</td>
<td>0.331±0.035a</td>
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<tr>
<td>llaB 10^9 CFU</td>
<td>0.252±0.014a</td>
<td>0.169±0.037a</td>
<td>0.184±0.045a</td>
<td>0.364±0.011b</td>
</tr>
</tbody>
</table>

*Means with the same letter are not significantly different (p<0.05).

were not significantly different at between 5 and 12 days after immunization (Table 4).

**DISCUSSION**

This study was performed to examine the safety and immunogenicity of attenuated S. enterica serovar Typhimurium llaB. The results showed that the virulence of S. enterica serovar Typhimurium llaB was effectively attenuated and had the protective effect against S. enterica serovar Typhimurium UK1, the challenging organism. Immunization with S. enterica serovar Typhimurium llaB elicited a higher immune response, including the increase of T- and B-cell proliferation in response to stimulation of mitogens (ConA and LPS), cytokine expression such as IL-2, -4, -6, and -10 in spleen, and serum antibody titer of IgG1, IgG2a, IgA, and IgM responding to the antigen of S. enterica serovar Typhimurium UK1.

Lymphocyte proliferation responses to mitogens are widely used to assess T- and B-cell functions [5, 14, 15]. Mice administered with an external antigen exhibited enhanced T- and B-cell functions, as indicated by elevated proliferation responses to the T-cell mitogen, Con A, and the B-cell mitogen, LPS. In the present study, spleen cells stimulated by Con A and LPS were more proliferating in the mice immunized with S. enterica serovar Typhimurium llaB than in the control mice, indicating that S. enterica serovar Typhimurium llaB increased both T- and B-cell functions.

T-cells are the main effectors and regulators of cell-mediated immunity [5]. On activation by antigen or pathogen, T-cells synthesize and secrete a variety of cytokines that serve as growth, differentiation, and activation factors for other immunocompetent cells. Recent studies have shown that T-cells can be divided into two functional types, Th1 and Th2, based on their cytokine profile [2, 4]. Th1 cells secrete IFN-γ, IL-2, and TNF-β, whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-10 [14]. Th1 cells are efficient inducers of cell-mediated immunity, including activation of macrophage and CTL, while Th2 cells provide better help for B-cell responses, including those of IgG1, IgE, and IgA isotypes. Generally, S. enterica serovar Typhimurium has been shown to induce a Th1-dependent immune response [14]. Both Th1 and Th2 cells may regulate IgA expression, and the Th2 cell subset has been shown to be more efficient. In the present study, RT-PCR analysis showed that administration of S. enterica serovar Typhimurium llaB to mice led to significant increase of IL-2, IL-4, IL-6, and IL-10 mRNA expressions in the spleens. These results mean

Table 4. Semi-quantitative RT-PCR analysis for IL-2, IL-4, IL-6, and IL-10 from spleen samples of mice immunized with 10^8 or 10^9 CFU of S. enterica serovar Typhimurium llaB at 5 and 12 days after immunization.

<table>
<thead>
<tr>
<th></th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
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</tr>
<tr>
<td>Control</td>
<td>0.929±0.086b</td>
<td>0.951±0.077b</td>
<td>0.849±0.068b</td>
<td>0.844±0.087b</td>
</tr>
<tr>
<td>llaB 10^8 CFU</td>
<td>1.218±0.096b</td>
<td>1.209±0.082b</td>
<td>1.114±0.097b</td>
<td>0.141±0.088b</td>
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<tr>
<td>llaB 10^9 CFU</td>
<td>1.375±0.097b</td>
<td>1.326±0.085b</td>
<td>1.239±0.075b</td>
<td>1.218±0.071b</td>
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<tr>
<td>12 days</td>
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<tr>
<td>Control</td>
<td>0.859±0.109b</td>
<td>0.838±0.107b</td>
<td>0.730±0.096b</td>
<td>0.708±0.082b</td>
</tr>
<tr>
<td>llaB 10^8 CFU</td>
<td>1.058±0.096e</td>
<td>1.169±0.104e</td>
<td>1.123±0.105e</td>
<td>1.302±0.107e</td>
</tr>
<tr>
<td>llaB 10^9 CFU</td>
<td>1.289±0.097e</td>
<td>1.197±0.074e</td>
<td>1.303±0.092e</td>
<td>1.442±0.129e</td>
</tr>
</tbody>
</table>

*Results were expressed as the density values of each cytokine divided with those of β-actin.

*Means with the same letter are not significantly different (p<0.05).
that that *S. enterica* serovar Typhimurium *ltaB* induced both Th1 and Th2 immune response in mice.

In conclusion, *S. enterica* serovar Typhimurium *ltaB* is an effectively attenuated strain originating from *S. enterica* serovar Typhimurium UK1 and has prominent immunogenicity. Therefore, this strain can be used as an attenuated live oral vaccine against *Salmonella* infection.

REFERENCES


