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A novel approach for construction of an inactivated typhoid vaccine candidate that effectively augments both humoral and cellular immune responses

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ABSTRACT

Salmonella enterica serovar Typhi ghost was constructed as a vaccine candidate against typhoid fever. An *asd*$^{+}$ plasmid pJHL187 harboring a ghost cassette comprised of PhiX 174 *E* lysis gene stringently controlled under the convergent promotor components and was transformed into the *asd* gene-deleted mutant *S.* Typhi Ty21a strain (STG). Twenty female BALB/c mice randomly assigned into two groups were subcutaneously vaccinated at 5 weeks of age to assess immunogenic characteristics of the constructed STG. The level of serum IgG in the immunized mice was significantly increased during the observational period ($P < 0.001$) as the mice showed the significant elevation of secretory IgA at week 6 compared to those in the non-immunized mice ($P < 0.05$). The $CD3^{+}CD4^{+}$ T cell subpopulation in the primed splenocytes showed approximately twofold increase in the immunized group. Further, the gene expression of various immunomodulatory cytokines associated with Th-1, Th-2 and Th-17 immunity was observed in *in vitro* restimulated splenocytes isolated from the immunized mice. Serum Bactericidal activity of antibodies produced in the rabbits immunized with STG was proved by the elimination of almost all of wild-type *S.* Typhi in the presence of exogenous complement over an hour at week 6 after the first immunization. The immuno-stimulatory traits of STG demonstrated that the construct effectively enhanced the immunological responses, providing a potential of STG as the vaccine candidate against typhoid fever.

1. Introduction

Typhoid fever caused by *Salmonella enterica* serovar Typhi presents a global health burden especially immunocompromised children living in developing countries with limited food hygiene and sanitation infrastructure [1–3]. *S.* Typhi, a human-restricted pathogen, is estimated to cause around 33 million cases of typhoid fever and a half million death annually in the world [4]. Particularly, infants and toddlers are also infected by this disease, followed by a high rate of complication and morbidity. Treatment with antibiotics is limited due to the emergence of multidrug–resistant *S.* Typhi in developing countries [5,6]. Two licensed typhoid vaccines, the parenteral Vi polysaccharide (Vi) (Typherix®or Typhim Vi®) and oral live attenuated Ty21a (Vivotif®) are currently available and recommended to use. Despite the presence of these approved vaccines, the vaccines have been used for travelers to affected countries rather than residents living in the endemic areas due to a limited health budget in developing countries, resulting in an inappropriate control of typhoid fever [7]. Based on last three years' data base, the recent systemic review reported that Vi vaccine based on Typhoid Vi antigen and Ty21a vaccine conferred 30–70 percent of protective efficacy [8]. In addition, licensed vaccine is not available for usage in children less than 2 years. A new conjugate vaccine, a conjugate of Vi polysaccharide bound to nontoxic exoprotein A of *Pseudomonas aeruginosa* (Vi-rEPA) is prepared [9], and PedaTyph™ vaccine, other conjugate vaccine developed by binding Vi to tetanus toxoid is currently licensed in India [10]. However, in the area where Vi based vaccine is used, a high background level of Vi antigen-specific IgG level make it difficult to differentiate vaccinated to infected residents when anti-Vi antibody assay is performed for an investigation of the prevalence [11]. Furthermore, Vi based vaccine has a lack of booster effect because of failure to mounting T cell related immune responses [2]. Thus, introduction of a new concept of vaccine is needed to reduce cost, and to improve immunogenicity, storability of the existing vaccines in the endemic areas.

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The bacterial ghost (BGs) system has developed as a novel inactive vaccine platform using the ΦX174 lysis E gene [12]. The lysis gene leads to form transmembrane holes in gram-negative bacteria. Subsequently, the contents are expelled during the lysis but intact bacterial envelope structures remain, which preserve antigenic conformational epitopes [13,14]. In development history of vaccine for typhoid fever, parenteral phenol-preserved or heated-inactivated whole cell vaccine was introduced. However, their continuing use was hampered by severe side effects caused by the overt reactogenicity [15]. In contrast, in ghost vaccine system Mader et al. indicated that bacterial ghost expressed all surface antigenic determinants including the most epitopes of a living cell [16]. In addition, the research group also revealed that both *E. coli* O26:B6 and *Salmonella* Typhimurium ghost did not cause any significant pyrogenic activity in the rabbit immunized with relatively high dose (250 ng per kg) of the ghost cells [16]. In the context of safety and practicality of ghost vaccine by expression of a lethal lysis gene *E* under inducible promoters, several gram-negative bacteria have been successfully prepared for BGs for the development of a human vaccine against *Escherichia coli* O157:H7, *Vibrio cholera*, *Helicobacter pylori*, and *Chlamydia trachomatis* [17–21]. Thus, the BGs could replace the use of the live attenuated or polysaccharide capsule vaccines against typhoid fever since a ghost construction of *S.* Typhi can retain the same entire surface antigenic determinants as their living counterparts, including lipopolysaccharide, capsular, and flagella etc [22,23] and may efficiently induce immunogenicity against these intact antigenic components.

This study describes a *Salmonella* Typhi ghost (STG) was constructed and this revealed exploiting systemic, mucosal and cellular immune responses in a mouse model. The serum bactericidal assay to evaluate functional antibodies generated by the immunization was performed and it showed the potential of elimination of the pathogen circulated in the host. This study is the first report of introduction of ΦX174 lysis gene *E* into *S.* Typhi, and preparation for an inactivated vaccine candidate against typhoid fever.

2. Materials and methods

2.1. Ethics statement

All animal experimental procedures were approved (CBNU2015-00085) by the Chonbuk National University Animal Ethics Committee in accordance with the guidelines of the Korean Council on Animal Care and Korean Animal Protection Law.

2.2. Bacterial strains, plasmids

Bacterial strains, plasmids, and primers utilized in the current study are described in Table 1. Strains carrying the ghost plasmid were incubated in the presence of 0.2% L-arabinose and the *asd* gene deleted strains were grown at 37 °C in LB broth or Brilliant Green Agar (BGA) supplemented with 50 μg/ml diaminopimelic acid (DAP). All bacterial strains were stored at −80 °C in LB broth containing 20% glycerol.

2.3. Construction and production of Salmonella Typhi ghost

Salmonella enterica serotype Typhi Ty21a strain JOL1497 which carries no plasmid was used to construct the ghost strain. The aspartate β-semialdehyde dehydrogenase (*asd*) gene-deleted mutant of *S.* Typhi Ty21a strain JOL1498 originated from JOL1497 was prepared by using the allelic exchange as previously described [24]. The JOL1498 was transformed with the plasmid pJHL187 by means of electroporation and the resultant strains were designated *S.* Typhi JOL1561. A single colony of JOL1561 was cultivated in 50 ml of nutrient broth (NB) with 0.2% L-arabinose at 27 °C until mid-logarithmic phase. The cells were collected, washed twice with NB and resuspended in 50 ml of NB without 0.2% L-arabinose at 42 °C in a shaking incubator at 200 rpm to induce the expression of lysis gene *E*. After 48 h, the bacterial cells were harvested via centrifugation for 10 min at 4000 rpm and resuspended in 1 ml of sterile phosphate-buffered saline (PBS). The expression of the gene *E* in JOL1561 ghost was validated by western blot analysis as previously described [25]. To ensure the absence of living cells and remove the remaining risk of its use as a vaccine strain, chemically induced lysis mediated by sodium hydroxide (NaOH) treatment was employed to prepare the ghost vaccine strain. The concentration of sodium hydroxide was determined using the minimum inhibitory concentration for effective lysis [26]. The 1.25 mg/ml NaOH was added onto the culture of lysed ghost bacterial cell mediated by *E* gene for 48 h and incubated for 5 mins at 37 °C to completely inactivate the lysed cells and were subsequently washed three times with sterile PBS. Complete lysis of the ghost cells was verified by counting the number of viable cells and by measuring optical density (OD at 600 nm wavelength). The final cell pellets were resuspended in autoclaved PBS and stored at −20 °C until use. Morphological features of the lysed JOL1561 ghosts was examined by scanning electron microscopy (SEM) as previously described [27]. Alteration of the ghost cell surface proteins following the lysis procedures was also assessed by western blot analysis followed by the protocol in the previously published using a rabbit polyclonal antibody raised against STG [25].

Table 1
Bacterial strains and plasmids used in this study.

Strain, plasmid	Description	Reference or source
Bacterial strains		
E. coli		
JOL232	F⁻ λ⁻ ϕ80 Δ[*lacZYA-argF*] *endA1 recA1 hadR17 deoR thi-1 glnV44 gyrA96 relA1 ΔasdA4*	Lab stock
S. Typhi		
JOL380	*Salmonella* Typhi wild-type from human	Lab stock
JOL1497	*Salmonella* Typhi Ty21a	Lab stock
JOL1498	Δ*asd*, a derivative of *Salmonella* Typhi Ty21a	This study
JOL1561	JOL1498 containgpJHL187	This study
Plasmids		
pJHL187	*asd*⁺ vector, p15A ori plasmid carrying ss *ompA*/His_6, multiple cloning site, *cI857*/λ*PR* promoter, araC P_{araBAD}, *phi*X174 lysis gene *E*	[35]

2.4. Immunization

A total of 20 female BALB/C mice at 5 weeks of age were divided into two groups. Group A and B mice were subcutaneously vaccinated with 100 μl of a suspension containing 1×10^8 CFU of JOL1561 or sterile PBS, respectively at week 0 and 2. At week 0, 2, 4 and 6 post-immunization (PI) in all groups, sera were obtained by centrifuging blood collected from orbital sinus of the mice and vaginal washes with PBS were sampled from the mice. For the subsequent immunological analysis using the primed splenocytes, additional ten mice (each five mice per group) were vaccinated with JOL1561 ghost cells following the same protocol described above at week 0. At week 2 after the first immunization, the splenocyte was aseptically isolated for the further processes. All the mice experiments performed in this study in duplicate and repeated twice.

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