Systemic immune responses to oral administration of recombinant attenuated \textit{Salmonella typhimurium} expressing \textit{Helicobacter pylori} urease in mice

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\textbf{AIM:} To evaluate whether attenuated \textit{Salmonella typhimurium} producing \textit{Helicobacter pylori} (H pylori) urease subunit B (UreB) could induce systemic immune responses against \textit{H pylori} infection.

\textbf{METHODS:} Attenuated \textit{S. typhimurium} SL3261 was used as a live carrier of plasmid pTC01-UreB, which encodes recombinant \textit{H pylori}UreB protein. Balb/c mice were given oral immunization with two doses of SL3261/pTC01-UreB at a 3-wk interval. Twelve weeks after oral immunization of mice, serum IgG antibodies were evaluated by ELISA assay. Gamma interferon (IFN-\(\gamma\)) and interleukin 10 (IL-10) in the supernatant of spleen cell culture were also assessed by ELISA.

\textbf{RESULTS:} After oral immunization of mice, serum specific IgG antibodies against UreB in vaccine group were much higher than that in PBS and native \textit{Salmonella} SL3261 control groups (\(A_{450} = 0.373 \pm 0.100 \) vs \(0.053 \pm 0.022, 0.142 \pm 0.039\), respectively, \(P<0.01\)). Moreover, IFN-\(\gamma\) in vaccine group was on average 167.53 \pm 29.93 pg/mL, which showed a significant increase vs that of PBS control group (35.68 \pm 3.55 pg/mL, \(P<0.01)\). There was also a tremendous increase of IL-10 in vaccine group compared to PBS and SL3261 control groups (275.13 \pm 27.65 pg/mL vs 76.00 \pm 7.15 pg/mL, 68.02 \pm 15.03 pg/mL, respectively, \(P<0.01)\). In addition, no obvious side effects in mice and no change in gastric inflammation were observed.

\textbf{CONCLUSION:} The multiple oral immunizations with the attenuated \textit{S. typhimurium} expressing \textit{H pylori}UreB could induce significant systemic immune responses, suggesting it may be used as oral vaccine against \textit{H pylori} infection.

\textbf{Key words:} \textit{Helicobacter pylori}, \textit{Salmonella typhimurium}; Vaccine; Systemic immune

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\textbf{INTRODUCTION}

\textit{Helicobacter pylori} (H pylori) is one of the most common bacteria worldwide, which infects more than 50% of the human population[1]. It is generally recognized that \textit{H pylori} infection is a major etiological factor in chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric B-cell lymphoma (MALT). The current therapy, based on the use of a proton-pump inhibitor and antibiotics, is efficacious but faces many problems such as patient compliance, possible recurrence of infection, complex dosing, costs and various side effects, and most importantly, development of antibiotic resistance. These compromise widespread clinical use. As a consequence, new strategies for the prevention and eradication of \textit{H pylori} infections are being explored. Vaccines are an attractive option, because they are both effective and economic in use. It is widely accepted that, given the worldwide prevalence of \textit{H pylori} infection, vaccination would be a preferable strategy[2]. A number of trials indicate that attenuated \textit{Salmonella typhimurium} strains can be used to deliver foreign antigens[3]. So, we had established recombinant attenuated \textit{S. typhimurium} SL3261 expressing \textit{H pylori} urease B subunit (UreB), which could elicit strong mucosal immune responses[4]. The purpose of the present study was to determine whether it could induce specific immune responses and whether it could be used as oral vaccine against \textit{H pylori} infection.

\textbf{MATERIALS AND METHODS}

\textbf{Animals}

Four to six weeks old female Balb/c mice were purchased from the Animal Resources Center, Fourth Military Medical University, Guangzhou. The animals were fed on a commercial diet and given water ad libitum.

\textbf{Bacterial strains, media, and growth}

The attenuated \textit{S. typhimurium} SL3261 (\textit{S. typhimurium} WARY hisG 46 aroA del 407 Fusaricres, etc.) kindly provided by...
H pylori

Analysis of anti-urease antibodies in mouse sera by immunoblotting

The presence of gamma interferon (IFN-$\gamma$) and interleukin 10 (IL-10) in the supernatant was detected by ELISA. The presence of gamma interferon (IFN-$\gamma$) and interleukin 10 (IL-10) in the supernatant was detected by ELISA. The presence of gamma interferon (IFN-$\gamma$) and interleukin 10 (IL-10) in the supernatant was detected by ELISA.

Cytokine measurement

The supernatant of spleen cell culture was collected. The supernatant of spleen cell culture was collected. The supernatant of spleen cell culture was collected.

Histology

Biopsy specimens from the antrum and the corpus were fixed in 10% buffered formalin, and 4-μm sections were cut. Sections were stained with HE to grade gastritis.

Statistical analysis

The differences in anti-UreB IgA and IgG antibodies as well as IFN-$\gamma$ and IL-10 from immunized and non-immunized mice were detected by sandwich ELISA.

RESULTS

Serum anti-UreB IgA antibodies in protected mice

As indicated in Table 1, the multiple oral immunizations with SL3261/pTC01-UreB could induce significant H pylori-specific serum IgG responses (P<0.01) (Table 1).

| PBS group | SL3261 group | Vaccine group |
|-----------|--------------|--------------|
| Serum IgG | 0.03±0.022   | 0.14±0.039   | 0.37±0.100* |

Table 1 Detection of intestinal IgA and serum IgG (A$_{max}$, mean±SD)

Measurement of cytokine IFN-$\gamma$ and IL-10

The difference in IFN-$\gamma$ and IL-10 between immunized mice and non-immunized mice (PBS) was significant (P<0.01). Although the difference in IL-10 between mice immunized with strain SL3261 alone and strain SL3261/pTC01-UreB was also significant (P<0.01), there was no significant increase of IFN-$\gamma$ between SL3261 group and SL3261/pTC01-UreB group (Table 2).

Table 2 Measurement of IFN-$\gamma$ and IL-10 (pg/mL, mean±SD)

Detection of anti-UreB antibodies in mice immunized with the salmonella vaccine strain

The protein of 61 kDa, corresponding to UreB, was recognized by serum from the vaccine group mice. This band was not detectable in strips tested from non-immunized mice or mice immunized with Salmonella only. But other non-specific bands were also observed in most lanes.

Histology of mice stomach tissue

Histology showed that the grade of gastritis had no difference between the vaccine group and control groups.

Observation of general character

In the initial stage of immunization, the mice had a jaded appetite and weight loss. No diarrhea and death occurred in immunized mice.

Recombinant attenuated S. typhimurium SL3261/pTC01-UreB stability

After 80 generations of continuous culture, nearly 100% of the SL3261/pTC01-UreB bacterial colonies were chloromycetin resistant and could express UreB protein.

DISCUSSION

Since the idea of vaccine against H pylori infection was raised in 1990, scientists have placed emphasis on the study of H pylori protein vaccine. Urease is necessary for colonization of H pylori and expressed at high levels by all H pylori strains. Furthermore, because of the high immunogenicity and the high conservation (98%) between different H pylori strains,
the urease is regarded as a promising candidate of \textit{H pylori} vaccine. Studies indicated that oral immunization of natural or recombinant UreB in combination with \textit{Cholera} toxin (CT) or \textit{Escherichia coli} labile toxin (LT) could protect mice from \textit{H pylori} infection\cite{1}. But in all successful vaccination protocols, mucosal adjuvants, i.e., CT or LT, are necessary. One major drawback with these bacterial adjuvants is that they are toxic in humans\cite{2}. This restricts the use of oral protein vaccine. Thus, the development of new vaccine strategies for \textit{H pylori} is indispensable. Live attenuated \textit{S. typhimurium} vaccine strains expressing foreign antigens are a promising new generation of vaccines that induce remarkably strong and specific immune responses in the mammalian hosts when given by mucosal immunization routes such as the oral, nasal, rectal and vaginal routes. Moreover, live \textit{S. typhimurium} used as vector for heterologous antigens do not require antigen purification, and they not only can protect antigen from degradation and denaturation in stomach but also express adjuvant activity that prevents induction of oral tolerance. \textit{S. typhimurium} SL3261 is an araA gene mutant that is invasive yet nonvirulent. Human trials indicate that attenuated \textit{S. typhimurium} strain is well tolerated and highly immunogenic and may be useful for the delivery of foreign antigens and immunoprotection against a variety of pathogens including \textit{H pylori}. So \textit{S. typhimurium} SL3261 is an efficient live bacterial vector\cite{3}.

The expression of protective immunity against gut pathogens is normally dependent both on local (mucosal) and systemic mechanisms\cite{4}. We cloned successfully the prokaryotic vector pTC01-UreB and established attenuated \textit{S. typhimurium} SL3261/pTC01-UreB expressing UreB subunit. We have shown that the multiple oral immunizations with SL3261/pTC01-UreB could elicit significantly \textit{H pylori}-specific mucosal IgA response\cite{5}. The increases in serum IgG and mucosal IgA anti-UreB antibodies indicate that UreB delivered by \textit{Salmonella} is highly immunogenic and capable of inducing specific humoral immunity and mucosal immunity.

At present, it is generally acknowledged that protective immunization has been associated with a progressive disappearance of Th1 cells and the development of a Th2 response\cite{6}. In a recent study, Guy and coworkers showed strong Th1 and Th2 responses elicited better protection than a predominant Th2 type response only. They thought that an appropriate balance between Th1 and Th2 type responses is required to achieve complete protection\cite{7}. SL3261/pTC01-UreB was vaccinated through the route of mucosal administration. Mucosal tissues favor the development of Th2-type responses\cite{8}. Our results showed that there was significant increase of IFN-γ and IL-10 in SL3261/pTC01-UreB group. These suggest that SL3261/pTC01-UreB can induce strong Th1 and Th2 responses. Nevertheless, the level of IFN-γ had no difference between SL3261/pTC01-UreB group and SL3261 control group. And the level of IFN-γ in SL3261 control mice was also higher than in PBS control mice. These facts demonstrate that the strong Th1 response is associated with attenuated \textit{S. typhimurium} used as vaccine vector, because attenuated \textit{S. typhimurium} is a kind of invasive pathogens\cite{9,10}.

Genetic stabilization of foreign antigen expression is a crucial step in the development of an immunogenic vaccine strain. Our data showed that there was no pTC01-UreB plasmid loss in SL3261/pTC01-UreB after 80 generations of continuous culture. Moreover, expression of UreB from \textit{S. typhimurium} SL3261/pTC01-UreB was also obtained at different phases of growth including in the stationary phase. All these clarify that the recombinant plasmid pTC01-UreB is stable in SL3261 and has no obvious toxicity. In addition, the facts that no obvious side effects for mice and no change in gastric inflammation were observed indicate \textit{S. typhimurium} SL3261/pTC01-UreB is safe. Our results show that the attenuated \textit{S. typhimurium} expressing \textit{H pylori} UreB may be used as oral vaccine against \textit{H pylori} infection. Henceforth, we need to consummate anti- \textit{H pylori} \textit{S. typhimurium} vaccine and evaluate its effect.

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