Effect of Deletion of Gene Cluster involved in Synthesis of Enterobacterial Common Antigen on Virulence and Immunogenicity of Live Attenuated Salmonella Vaccine when Delivering Heterologous Streptococcus pneumoniae Antigen rPspA

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Abstract

Background: Enterobacterial common antigen (ECA) is a family-specific surface antigen shared by all members of the Enterobacteriaceae family. Previous studies showed that the loss of ECA results in *Salmonella* attenuation, indicating its usefulness as a vaccine candidate for *Salmonella* infection, but no studies have shown whether the mutation resulting from the deletion of the ECA operon in conjunction with other mutations could be used as an antigen vehicle for heterologous protein antigen delivery.

Results: In this study, we introduced a nonpolar, defined ECA operon deletion into wild-type *S. Typhimurium* χ 3761 and an attenuated vaccine strain χ 9241, obtaining two isogenic ECA operon mutants, namely, χ 12357 and χ 12358, respectively. A number of *in vitro* and *in vivo* properties of the mutants were analyzed. We found that the loss of ECA did not affect the growth, lipopolysaccharide (LPS) production and motility of *S. Typhimurium* wild type strain χ 3761 and its attenuated vaccine strain χ 9241 but significantly affected the virulence when administered orally to BALB/c mice. Furthermore, the effects of the ECA mutation on the immunogenicity of a recombinant *S. Typhimurium* vaccine strain χ 9241 when delivering the pneumococcal antigen rPspA were determined. The result showed that the total anti-PspA IgG level of χ 12358 (pYA4088) was slightly lower than that of χ 9241 (pYA4088), but the protection rate was not compromised.

Conclusions: ECA affects virulence and benefits the Th2 immunity of *Salmonella Typhimurium*, therefore, it is feasible to use a reversibly ECA mutant mode to design future *Salmonella* vaccine strains for heterologous protective antigens.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

ECA operon deletion in *S. Typhimurium* χ 3761 A. Map of the ECA operon. B. Schematic strategy for deletion of the ECA operon by allelic exchange using suicide vectors; the whole ECA operon from *wecA* to *wecG* was deleted. C. ECA operon deficiency mutation identification by PCR amplification. Primer pairs for Doperon-1F/Doperon-2R were used to amplify the ECA operon using the ECA-deficient *S. Typhimurium* mutant χ 12357 (lane 1) and the wide type χ 3761 (lane 2) as the PCR template. A PCR product of approximately 800 bp was observed for χ 12357, but no bands were observed for wild type *S. Typhimurium* χ 3761. D. Detection of ECA using ECA-specific mAb898 antibody by western blotting. The wide type *S. Typhimurium* χ 3761 could produce ECA, but the ECA-deficient *Salmonella* strain χ 12357 lost the ability to produce ECA.

Figure 2

Phenotypes of the wide type *S. Typhimurium* and ECA-defective mutant strain. A. Growth curves. Overnight cultures were diluted to an optical density of 0.025 and incubated at 37°C with shaking at 180 rpm; the OD600 was measured at the appropriate time. B. Bile sensitivity assay. Three-microliters of a 10-fold dilution of the overnight cultures of the wide type χ3761 and mutant χ12357 strains were deposited and incubated for 12 h at 37°C in LB plates with or without 1% DOC. C. LPS profiles. LPS from χ3761 and the mutant χ12357 were subjected to 12% SDS-PAGE and then silver stained.

Figure 3

Bacterial colonization in mouse organs. Groups of mice (3 mice/group) were orally inoculated with approximately 1×10^9 CFU of the indicated strains. The bacterial loadings (log CFU/g) in the tissue were determined on days 3, 6, 9, and 12 post-inoculation in the liver (A), spleen (B), and PP (C). The horizontal lines represent the means, and the error bars represent the standard errors of the means.

Figure 4

Serum responses and cross-reactivity Total serum IgG specific for rPspA (A), IgG1 specific for rPspA (B) and IgG2a specific for rPspA (C) in mice immunized with χ 9241 (pYA4088) or χ 12358 (pYA4088) were measured according to standard antibodies by ELISA. The error bars represented variations between triplicate wells. Mice were boosted at week 4. ***, $P < 0.01$ compared to the concentration from the mice immunized with χ 9241 (pYA3493). ##, $P < 0.01$; ###, $P < 0.001$ compared to the concentration from the mice immunized with χ 9241 (pYA4088).

Figure 5

Serum responses and cross-reactivity IgG levels of sera obtained from mice orally immunized with χ_{9241} (pYA4088) or χ_{12358} (pYA4088) to homologous and heterologous SOMP were measured. The error bars represented variations between triplicate wells. Mice were boosted at week 4. ***, $P < 0.01$ compared to the concentration from the mice immunized with χ_{9241} (pYA3493). ##, $P < 0.01$; ###, $P < 0.001$ compared to the concentration from the mice immunized with χ_{9241} (pYA4088).

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