Comparison of Immune Responses Induced by Recombinant Attenuated *Salmonella typhi* Carrying Eukaryotic Expression Plasmid or Prokaryotic Expression Plasmid of HCV Core Protein

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**Abstract**

Hepatitis C virus (HCV) core protein is considered to be an attractive candidate for development of protective HCV vaccines. However, this protein may attenuate the induction of systemic immune responses due to its immunomodulatory properties. In this study, we constructed a HCV core gene-containing eukaryotic expression plasmid pCl-C and an in vivo-
inducible prokaryotic expression plasmid pZW-C and transformed the recombinant plasmids into an attenuated *Salmonella typhimurium aroA* strain SL7207. The resulting bacterial strains SL7207/pCI-C and SL7207/pZW-C were used to orally immunize BALB/c mice and the immune responses specific to HCV core protein were assessed. Immunization with the recombinant bacteria SL7207/pCI-C led to a persistent drop in percentage of CD3+ CD4+ T cells and induced a weak anti-core IgG production. Splenocytes from SL7207/pCI-C immunized mice developed a relatively weak proliferation response and inferior cytotoxic activity compared to those from the mice immunized with bacteria SL7207/pZW-C. Boost immunization with SL7207/pCI-C yielded limited improvement in immune strength while the boost with bacteria SL7207/pZW-C significantly enhanced the immune response. These results suggest that de novo synthesis of native HCV core protein may blunt the induction of immune responses. Attenuated *S. typhimurium* carrying HCV core protein could efficiently activate systemic cellular and humoral responses and may be a promising strategy for the development of core-based HCV vaccines.

**Key words**  Hepatitis C virus core protein attenuated *S. typhimurium* vaccine immune responses

1 1.2 HCV J4 cDNA PCR HCV J4 cDNA PCR HCV J4 cDNA PCR HCV J4 cDNA PCR

HCV J4 cDNA PCR HCV J4 cDNA PCR HCV J4 cDNA PCR HCV J4 cDNA PCR

1.3 HCV 293 T pCI-C pCI-neo 293 T 293 T

293 T 293 T 48 h Western blot HCV HCV HCV HCV

Biodesign

1.4 HCV pZW-C pZV pZV pZV pZV

pZV pZV pZV pZV pZV

SL7207 SL7207 SL7207 SL7207 100 mg/L

80 mmol/L MgCl2 LB 37 °C 80 mmol/L MgCl2 0 ~ 100 mmol/L MgCl2

LB 37 °C 3 h Western blot HCV HCV
1.5 pCI-C[pCI-neo] SL7207 SL7207/pCI-C SL7207/pCI-neo 80 mmol/L MgCl₂ 100 mg/L LB 6 18 BALB/c BALB/c 1 x 10⁶ T cells 1 4

1.6 ELISA HCV IgG

1.7 T CTL 12 % D 3 CD3⁺ CD4⁺ CD3⁺ CD8⁺ T PE CD4⁺ CD8⁺ BD Pharmingen

1.8 T CTL 96 % D 3

2.1 HCV 293T pCI-C 293T HCV 22 kD 1 nmol/L MgCl₂ HCV 100 mmol/L MgCl₂ LB 293T HCV 293T

2.2 HCV IgG SL7207/ pZW-C SL7207/pCI-C SL7207/pCI-neo SL7207/pCI-C

2.3 T CD3⁺ CD4⁺ CD3⁺ CD4⁺ T 3 3 SL7207/pCI-C

HCV 20 μg/ml 50 μg/ml 72 h MTS stimulation index SL7207/pCI-C HCV F815 CTL

1.9

2 Western blot pCI-C 293T HCV 22 kD 1 nmol/L MgCl₂ HCV 100 mmol/L MgCl₂ LB HCV 293T HCV

2.4 T HCV SL7207/pCI-C SL7207/pCI-neo SL7207/pCI-C SL7207/pZW-C

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2.5 CTL

![Diagram](image)

**Fig. 3** Analysis of CD3+ CD4+ T cells by flow cytometry. Asterisks indicate a significant difference compared to the control group (P < 0.05).

**Fig. 4** The T lymphocyte proliferative response of immunized mice.

**Fig. 5** CTL response of immunized mice.

**A** Assay after the second immunization. **B** Assay after the fourth immunization.
分析表达的抗原与细菌残骸在抗原提呈细胞内被共同免疫对的进行抗原提呈，诱导免疫应答的途径与天然结构的密切关系。如而疫苗同时肌肉注射接种则能有效提高系统性免疫应答进行免疫接种并非可取的方式，而以外源性抗似，更有利于反应则发现，重组菌导的的负向调控作用更为敏感。检测小鼠的以及反应强度的限制可能是在细胞内的定位以及免疫调控作用密切相关，而胞因子，如其他类似的病毒抗原，也许存在类似机制。本研究中构建的携带原核表达质粒的沙门菌因在宿主细胞便、成本低廉等优点，从而可望作为以核心蛋白为靶抗原的，与在细菌内表达抗原，无磷酸化修饰，3-4细胞增殖反应的影响并不明显。0细胞数量明显降低有关，该结果还提示，1细胞增殖应答与体液免疫应答，有与各自所诱而3-4和HCV、IL-2、IFN-γ、CD4⁺、CD8⁺、T⁺、CTL、SL2707/pCl-C、HCV、SL2707/pZW-C、IL-2、GM-CSF、DNA、SL2707/pCl-C、HCV、SL2707/pZW-C、HCV。REFERENCES


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