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LLO as a genetic adjuvant enhances hepatitis C virus NS3 DNA vaccine immunogenicity

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Introduction: Hepatitis C virus (HCV) chronic infection is a worldwide health problem which affects almost 3% of the world's population. To develop HCV vaccine, induction of potent T-cell response is consider to that target the immunogenic and conserved region. Listeriolysin O (LLO) of *Listeria monocytogenes* can be as an adjuvant to initiate T-cell immune responses. Herein, we cloned and expression fusion LLO-NS3 in mammalian cell line then evaluated T-cell immune response of recombinant DNA vaccine in mice model.

Methods: cDNA corresponding to partial immunogenic length of NS3 (rNS3, aa 1191-1380) was constructed by RT-PCR on RNA purified from HCV patients. rNS3 was cloned on pcDNA3.1-LLO and then recombinant plasmid evaluated by sequencing. Finally the plasmid was transfected to HEK293T cell line and fusion protein expression was confirmed by Western blotting. Mice were immunized three doses of the recombinant vector or pcDNA3.1-NS3 (as control group) and pcDNA3.1 (as negative control group) in 3-week intervals and their immune responses were evaluated using cytotoxic T-lymphocyte (CTL) activity by lactate dehydrogenase releasing method.

Results: Proper expression of the recombinant protein in the expected size (around 70 kDa) was confirmed using Western blotting. The immunization results indicated that cytotoxicity T-cell responses of vaccinated mice were significantly increased compared to control group ($p<0.05$).

Discussion: pcDNA3.1-LLO-NS3 demonstrated strong T-cell immunogenicity in a murine model. Our primary results demonstrated that truncated region of NS3 as immunogenic truncated region and LLO as genetic adjuvant can induce cell immune responses by activating the Th1 pathway.

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Successful activation of the behavioral drivers of the stakeholders involved in vaccine purchasing and usage

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The vaccine segment is anticipated to be one of the fastest growing one of the healthcare industry and several leading firms have stepped up vaccine investments in recent years. Unlike therapeutic agents, vaccines are administered to healthy individuals only once or very infrequently during a life time. Vaccines generate positive externalities, the ignorance of which by end-users may lead to resurgence of transmissible diseases and governmental interventions such as mandating vaccination. Bringing new vaccines to market requires carefully orchestrated programs targeting the multiple types of stakeholders along the entire value chain and addressing their respective purchasing behavior drivers. Against a backdrop of anti-vaccination buzz and vaccine fatigue, successful global launch and sustainable usage of a vaccine requires the development of a multi-pronged strategy addressing all aspects in relation to acceptability (e.g. the motivation to immunize despite the quasi-disappearance of the disease), accessibility (e.g. supply chain services), availability (e.g. mechanisms ensuring reliability of supply) and affordability (e.g. tiered pricing policy taking country differences in per capita income into account).

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