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## Characterization of recombinant vaccine constructed by individually cloning of HIV1 C gag, env and polRT genes using Semliki Forest virus vector

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The development of a safe, immunogenic, globally effective and affordable vaccine could be useful in control of HIV/AIDS. The recombinant vaccines developed by cloning of HIV genes using different vectors have not been found to be effective due to poor or moderate immunogenicity and/or safety. Semliki Forest virus (SFV), an alpha virus does not have pre-existing immunity, has cytoplasmic but no nuclear expression of heterologous proteins and are non-pathogenic in humans. Therefore, HIV1 Indian subtype C *gag, env and polRT* genes were individually cloned using SFV vector to generate recombinant SFV2gen replicon RNA constructs and subsequently generated recombinant SFV2gen viral like replicon particles (VRP) designated as rSFV2gen/gag VRP, rSFV2gen/env VRP, and rSFV2gen/polRT VRP by co-electroporation with Helper RNA. I*n vitro* studies demonstrated high levels of expression of respective HIV1 proteins and their localization in cytosol and not nucleus from all three recombinant constructs following infection of BHK-21 cells. The recombinant RNA constructs and VRPs individually and in combination of three constructs elicited significantly high cell mediated immune responses as detected by INF gamma and IL2 Assay and humoral immune responses in mice. VRPs have been found to be more immunogenic as compared to RNA constructs. Studies demonstrated that all three recombinant SFV2gen based vaccine constructs of Indian subtype C *gag, env and polRT* genes were highly immunogenic in the mice model and therefore promising candidate vaccine for control and management of HIV/AIDS.

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## Use of O-specific polysaccharides for preparation of immunogenic conjugates of Salmonella enterica serovars Typhi and Paratyphi A

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Typhoid and paratyphoid fevers, caused by *Salmonella enterica* serovars Typhi (S. Typhi) and Paratyphi A (S. Paratyphi A) are major health problems in developing countries. The available vaccines have certain limitations regarding their efficacy and inability to induce an immune response especially in individuals under 2 years of age. Conjugate vaccines which consist of a bacteria-specific polysaccharide chemically bound to a carrier protein overcome these problems by inducing a T-cell dependent immune response characterized by enhanced immunogenicity in all ages. Traditionally, Vi antigen of S. Typhi has been used for vaccine preparation but realization of importance of Vi negative isolates in recent years has made it imperative to prepare vaccines based on antigens which are present universally in all isolates. So we selected O-specific polysaccharides (OSP) for our experiments. In this study, O-specific polysaccharides (OSP) of S. Typhi were conjugated to diphtheria toxoid (DT) using adipic acid dihydrazide (ADH) as a linker. These conjugates (OSP-AH-DT) were then evaluated for their immunogenicity using mice as a model and showed significantly higher levels of IgG ELISA titers (P=0.0241 and 0.0245) than lipopolysaccharides alone. Different immunization schedules were compared and it was found that schedule-B (three injections with 4-weeks interval) induced higher immune responses than schedule-A (three injections with 2-weeks interval). We showed that diphtheria toxoid can be successfully employed as a carrier protein for conjugation with S. Typhi OSP and the resultant conjugates elicit adequate immune response. We carried out similar successful experiments by preparing conjugates of S. Paratyphi A OSP and diphtheria toxoid.

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