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Lipopeptide Nanoparticulate Vaccine Candidates for the Induction of Protective Immune Responses

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1. Introduction

The development of an effective vaccine for group A streptococci (GAS) has been challenged by the induced autoimmunity of epitopes derived from the C-repeat regions. Additionally, there are B-cell epitopes that have been shown to react with human heart tissue. Shorter safe B-cell epitopes, show little or no immunogenicity unless bound to a delivery platform including the conjugation to an inbuilt adjuvant. Self-adjuvanting lipid core peptide (LCP) systems where the antigen(s), carrier and adjuvant were within the same molecular entity has been developed. The LCP amphiphilic construct was incorporated into liposomes to produce particles with the desired size. The construct alone elicited high-levels of antibody titers comparable to that of the positive control (J8 + Complete Freund's adjuvant). The developed strategy to produce nanoparticles, consisting of a peripheral antigenic epitope layer conjugated to a dendrimer core, which is both self-adjuvanting and produces a strong immune response to the GAS M-protein, offers an attractive alternative to conventional vaccine approaches. The greatest advantage of this system being the generation of protective immune response after oral administration. Our dendrimer-nanoparticles vaccine approach should be readily acquiescent to other pathogenic organisms in addition to GAS, and may prove particularly useful for the design of vaccines against infection deceases know to stimulate autoimmune response in a host.

2. Materials and methods

Peptide (J14) and LCP-1 were synthesized on pMBHA rink amide solid resin using microwave-assisted (CME Corporation, NC, USA) solid-phase peptide synthesis under the standard Boc-based coupling protocol. The peptides were cleaved from resins, purified (HPLC) and characterized (MS).Trimethyl chitosan was synthesized by reductive methylation of chitosan with methyl iodide in a strongly alkaline environment at an elevated temperature.

3. Results

We synthesized vaccine candidates containing the dendritic structure (LCP) consisting of a lipoamino acids, poly-lysine carrier and a peripheral generation of the minimal B-cell epitope (J8) and CD4+ T helper cell epitope (P25). Liposomes which contained the LCP were formulated and optimized for charge and lipid content using a thin film formation method. These formulations were subsequently characterized. Immunological experiments were performed on mice immunised with vaccine candidate. Blood samples were collected and IgA and IgG titers were determined.

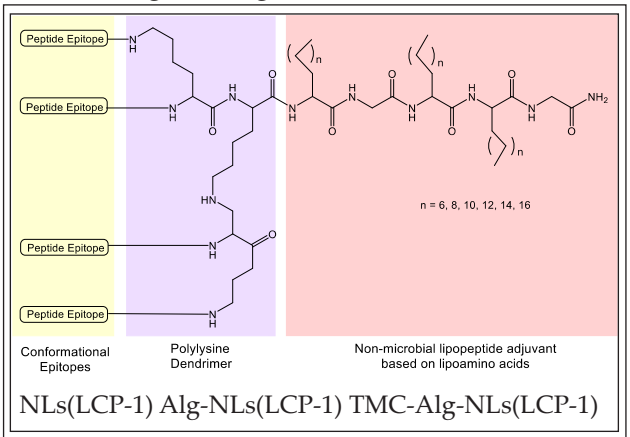

Figure 1 *General structure of the self-adjuvanting LCP system*

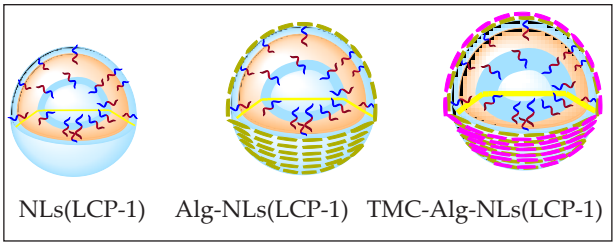

Figure 2 *Liposomes with entrapped LCP1 covered by layers of alginate and trimethyl-chitosane polymers.*

For oral administration the liposomes containing the LCP antigens were covered with an alginate polymer layer (for protection in acidic environment) then with a trimethyl-chitosan polymer layer (to protect in basic environment).

Mice were immunized with the constructs and high titer of IgG (blood samples) and IgA (saliva samples) titers were detected. Importantly, the TMC-Alg-NLs(LCP-1) group demonstrated high titers of IgG even at day 185 of post-primary immunizations. In contrast, salivary IgA response almost diminished at day 150. Thus, the mice upon immunization with the lead vaccine candidate, TMC- Alg-NLs(LCP-1), were able to maintain long-term systemic immunological response and partially prevail salivary IgA titers over the time (1,2). We also prepared different biodegradable polymer [dextran, poly-(lactic- coglycolic-acid) (PLGA), and poly-L-lysine] nanoparticles (NPs)-based delivery systems for LCP-1. The NPs were characterised by their size, charge, morphology, antigen-presenting cells (APCs) uptake and subsequent APCs maturations efficacy, followed by in vivo nasal immunisation in mice. Interestingly, positively-charged poly-L-lysine NPs were non-immunogenic, while negatively charged PLGA NPs induced similar responses to antigens adjuvanted with cholera toxin B (3). Additionally, the B-cell epitope J8, derived from GAS M protein, and universal T-helper Pan HLA-DR-binding epitope peptide (PADRE), were conjugated to poly (methyl acrylate) (PMA) to form a self-assembled nanoparticle vaccine candidate (PMA-P-J8). Strong systemic and mucosal immune responses were induced upon single oral immunization of mice with the conjugate. The antibodies generated were opsonic against GAS clinical isolates as measured after boost immunization. Thus, we developed a simple conjugate as an effective, adjuvant-free oral peptide-based vaccine (4,5).

4. Conclusions

The developed strategy to produce nanoparticles, consisting of a peripheral antigenic epitope layer conjugated to a dendrimer core, which is both self-adjuvanting and produces a strong immune response to the surface antigen, offers an attractive alternative to conventional vaccine approaches. The greatest advantage of this system, that can generate protective immune response after oral administration. Our nanoparticles vaccine approach should be readily acquiescent to other pathogenic organisms in addition to GAS, and may prove particularly useful for the design of vaccines against infection deceases know to stimulate autoimmune response in a host.

5. Acknowledgements

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