

## Liposaccharides in Peptide Vaccine Delivery

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### Introduction

Instability of conventional vaccines, the non-availability of carriers and adjuvants, and the potential danger of using live microorganisms have led to the development of synthetic vaccines [1]. The aim of this study was to develop a synthetic liposaccharide peptide-based vaccine to prevent group A streptococcal (GAS) infection. Infection with GAS can lead to many diseases, including pharyngitis, pyoderma, invasive diseases, rheumatic fever and rheumatic heart disease and is responsible for more than 500,000 deaths per annum [2]. Protection against GAS infection is mediated predominantly by opsonic antibodies directed against the surface M protein, the major virulence factor of GAS. Peptide epitopes (J8 and J14) used in this study contain a portion of the carboxy-terminal C-repeat region of this M protein, a conserved sequence in 70 % of GAS strains, thereby offering the capacity to create a vaccine which could potentially prevent up to 70% of GAS infections.

Carbohydrates provide multiple attachment points for peptides, and the conjugation of multiple copies of a single peptide to a carrier has been demonstrated to produce higher antibody responses compared to the administration of a single peptide epitope [3]. The attachment of synthetic lipids to peptide antigens has been shown to effectively increase the immune response to poorly immunogenic peptide antigens as well [4, 5, 6]. Therefore the conjugation of the lipid core peptide (LCP) system (adjuvant) with sugar units (carrier) represents one of the most important strategies being investigated in drug delivery.

Another approach of applying carbohydrates, lipids and peptide epitopes within one drug delivery unit is to use carbohydrates for receptor targeting. Cervical cancer is the second most common cancer in women worldwide (500,000 new cases each year, 250,000 deaths) with human papillomavirus (HPV) types 16 and 18 demonstrated to be the main causative agents. Currently over 100 different HPV genotypes have been identified, of these 40 are known to infect the genital tract, with about 14 types associated with cervical cancer. High risk HPV type 16 has been found to be responsible for approximately 50% of cervical cancers. HPV E6 and E7 proteins are continuously over-expressed in cervical cancer, and are believed to be responsible for HPV-induced cell transformation and cancer. Thus, the E6 and E7 proteins represent important targets for cervical cancer vaccine development. Mannose receptor targeting has the potential to enhance the immune response against antigens coupled to appropriate mannose

receptor ligands, as such mannose was incorporated into the vaccine.

### Results and Discussion

Glucopyranosyl and galactopyranosyl azides were cyanoethylated and converted into derivatives bearing linkers with terminal carboxylic acid functionalities at the anomeric position and four Boc-protected terminal amino groups.[7] These derivatives were attached to MBHA resin previously loaded with a lipodic adjuvant (C<sub>12</sub>-Gly-C<sub>12</sub>-C<sub>12</sub>-Gly; C<sub>12</sub> = 2-amino-D,L-dodecanoic acid). After Boc-deprotection, peptide sequences, J8 (QAEDKVKQSREAKKQVEKALKQLEDKVVQ) or J14 (KQAEDKVKASREAKKQVEKALEQLEDKVK) were coupled onto the carbohydrate scaffolds using stepwise solid-phase peptide synthesis to form novel vaccine systems. Finally, the products (**1** - **3**, Fig. 1) were cleaved from the resin using anhydrous hydrofluoric acid, then purified by RP-HPLC and characterized by LC-ESI-MS and RP-HPLC. As a control, a conventional lipid-core peptide system (**4**) was produced which contained four copies of the J8 peptide antigen (Fig. 1).

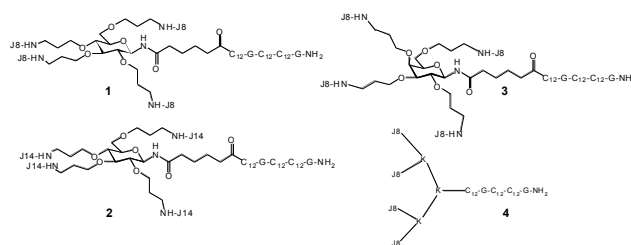


Fig. 1. Structure of vaccine candidates; D-glucose and D-galactose core constructs bearing J8 or J14 antigens (**1-3**) and poly-lysine core with J8 antigen (**4**).

*In vivo* experiments were performed in female B10.BR (H-2<sup>k</sup>) mice. The mice (n=5/group) were injected subcutaneously on days 0, 21, 31 and 41 with 30 µg of these immunogens **1** - **4**. Blood samples were collected one day before each injection and were analyzed by an enzyme-linked immunosorbent assay (ELISA) for total antigen-specific serum IgG antibodies elicited by immunization. The vaccine structure-immunity response relationship was examined by comparing candidates with different carbohydrate core (D-glucose or D-galactose) and peptide epitope (J8 or J14) in reference to poly-lysine core (**4**), see Figure 2. No significant difference was observed between the levels of J8-specific serum IgG antibodies elicited by carbohydrate core based vaccines **1** and **3** (administered without adjuvant) and the positive control

group (administered J8 mixed with CFA) which demonstrates the high potential for using carbohydrate cores in the design of future subunit vaccines (Fig. 2). Changing the carbohydrate core did not have an effect on IgG antibody levels but we expect to observe a difference in their ability to protect immunized mice against a challenge with GAS strains due to the difference in their peptide orientations. On the other hand, a little effect was observed due to changing the peptide epitope (Fig. 2).

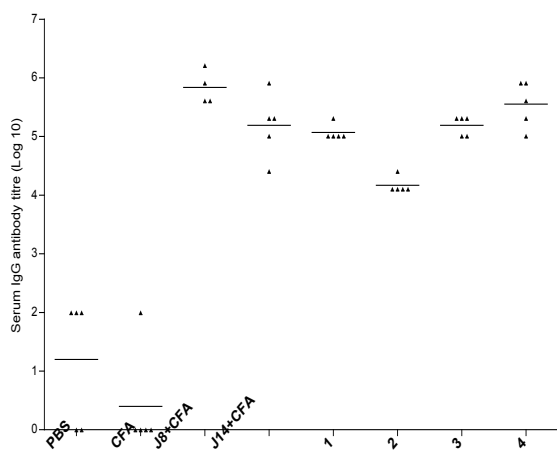


Fig. 2. J8 or J14 specific serum IgG antibody titers (log10) at the final bleed (day 50) elicited in response to immunization of B10.BR ( $H-2^k$ ) mice at the tail base with the carbohydrate core based vaccine candidates (1 - 3) and the poly-lysine core based vaccine (4), as determined by ELISA.

Novel vaccines against HPV-16 associated cervical cancer were also developed using a modified LCP system. LCP-systems were synthesized using stepwise solid-phase peptide synthesis on *p*MBHA resin using Boc-chemistry. The LCP-systems incorporated four copies of either the HPV-16 E7<sub>44-62</sub> peptide (QAEPDRAHYNIVTFCKCD), a randomized version of this peptide, or a chimeric peptide incorporating an ovalbumin cytotoxic lymphocyte and T-helper epitope (ISQAVHAAHAEINEAGRSIINFELK). Mannose, either in per-*O*-acetylated or non-acetylated forms, was conjugated to the N-terminus of these vaccines via a spacer (Fig. 3) to provide the opportunity for mannose receptor targeting. Three vaccines were synthesised, each containing four copies of the HPV-16 E7<sub>44-62</sub> peptide. Two of the vaccines featured four mannose residues to target dendritic cells, which have mannose-specific receptors.

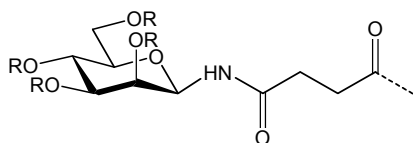


Fig. 3. Mannose (per-*O*-acetylated or non-acetylated; R = Ac or H) with a spacer which was used for conjugation to the LCP vaccines.

Immunological activity was assessed by immunizing C57BL/6 mice which were then challenged with TC-1 tumour cells. In general, mice immunized with HPV-16 E7<sub>44-62</sub> LCP-systems incorporating either per-*O*-acetylated or non-acetylated mannose residues, demonstrated no tumor growth. In comparison, mice immunized with HPV-16 E7<sub>44-62</sub> LCP-systems without attached carbohydrates demonstrated little or no tumor growth. This suggested that mannosylation was capable of increasing the capacity of E7<sub>44-62</sub> LCP-systems to clear or reduce the size of HPV-16 associated tumors. Mice immunized with the ovalbumin T-helper and cytotoxic lymphocyte epitope containing LCP-systems, the randomized E7<sub>44-62</sub> LCP systems, or phosphate buffered saline all developed TC-1 tumors following tumor challenge [8].

In conclusion, a novel drug delivery system combining lipoamino acids and carbohydrates has been developed and vaccine candidates (incorporating synthetic J8 or J14 peptide epitopes) against GAS infection were synthesized. Immunological studies demonstrated its capacity to induce antibody responses comparable to positive controls which were administered with CFA. Moreover, these constructs are suitable for use as a template for synthesis of other vaccines. The LCP-system was demonstrated to adjuvant the HPV-16 E7<sub>44-62</sub> peptide, as evidenced by reductions in TC-1 tumor size following tumor challenge. Conjugation of mannose, in both per-*O*-acetylated and non-acetylated forms, resulted in significantly smaller TC-1 tumor sizes compared with non-mannosylated analogues. Further research is required to determine if this was due to dendritic cell mannose receptor targeting.

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