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Structure–activity relationship of lipid core peptide-based Group A Streptococcus vaccine candidates

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ABSTRACT

Infection with Group A Streptococcus (GAS) can result in a range of different illnesses, some of which are fatal. Currently, our efforts to develop a vaccine against GAS focuses on the lipid core peptide (LCP) system, a subunit vaccine containing a lipoamino acid (LAA) moiety which allows the stimulation of systemic antibody activity. In the present study, a peptide (J14) representing the B-cell epitope from the GAS M protein was incorporated alongside a universal T-helper epitope (P25) in four LCP constructs of different spatial orientation or LAA lengths. Through structure–activity studies, it was discovered that while the alteration of the LCP orientation had a weaker effect on immunostimulation, increasing the LAA side chain length within the construct increased antibody responses in murine models. Furthermore, the mice immunised with the lead LCP construct were also able to maintain antibody activity throughout the course of five months. These findings highlight the importance of LAA moieties in the development of intranasal peptide vaccines and confirmed that its side chain length has an effect on the immunogenicity of the structure.

1. Introduction

Group A Streptococcus (*Streptococcus pyogenes* or GAS) is a Gram positive bacteria that can cause illnesses ranging from benign pharyngitis to invasive diseases like necrotising fasciitis and toxic shock syndrome. GAS-associated illnesses represent a global health problem, causing more than 600 million new cases of infection each year and resulting in a minimum of 500,000 deaths.[1,2] Delayed or inadequate treatment of GAS infections may also result in the development of post-infectious complications such as rheumatic fever (RF) and rheumatic heart disease (RHD), which are responsible for the majority of GAS-related mortality.[3] This highlights the need for the development of an effective prophylactic vaccine against GAS.

The development of a vaccine against GAS has primarily focused on the M-protein, a major virulence factor ubiquitous to all GAS strains.[4] Two areas of the M-protein present a particular research interest: the N-terminus and the C-repeat region.[5] The N-terminus is hypervariable and frequently undergoes genetic recombination, resulting in a rapid turnover of strains.[6–8] Due to this, antibodies induced against the N-terminus are serotype-specific and are only protective against particular strains of GAS, implicating the need to tailor vaccines for GAS endemic to different regions.[9] Conversely, the C-repeat region of the M-protein is highly conserved.[10] Epitopes derived from this region can overcome the restrictions regarding serotype specificity, allowing for the development of a vaccine that is protective against all GAS strains.

One such promising peptide epitope is p145 (LRRDLDASREAKKQVEKALE), which has been shown to generate protective antibodies capable of opsonising multiple serotypes of GAS.[10–14] However, immunisation with p145 poses a risk of generating T-cell autoimmune responses due to its sequence similarity to the human cardiac myosin.[15] Further research has identified J14 (KQAEDKVK***ASREAKKQVEKALE***QLEDKVK), a minimal peptide epitope that excludes the potentially deleterious T-cell sequences.[10] J14 is composed of 14 amino acids from p145 (sequence in bold) enclosed between two non-streptococcal sequences designed to maintain the α-helical conformation of a native epitope in the protein. It has been demonstrated that J14, when combined with the

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appropriate delivery system, can confer protective B-cell antibodies in mice against heterologous GAS strains.[10,16,17]

As a short peptides, J14 is immunologically inert on its own. Therefore, it is coupled with a self-adjuvanting moiety in a delivery system called a lipid core peptide (LCP). LCPs contain immune stimulating lipoamino acids (LAAs) which are α-amino acids with a long alkyl side-chain. The number of LAAs incorporated into the LCP, and the length of their side-chains, can be easily modified.[18] LAAs have demonstrated potent adjuvanting activity by activating toll-like receptor 2 (TLR2) on antigen presenting cells (APCs), leading to the induction of an immune response.[19]

The LCP system is able to induce protective immunity in both the mucosal and systemic immune compartments.[19,20] This permits delivery of LCP vaccines via the intranasal route. As GAS typically resides on the mucosal epithelium of the upper respiratory tract, intranasal vaccination benefits by its ability to stimulate both IgA and IgG antibodies.[21,22] IgA, the primary antibody on mucosal surfaces, inhibits GAS adhesion and colonisation, thus preventing bacterial dissemination that could lead to systemic infection.[21,23] However, IgA is inadequate as an opsonin and as an activator of the complement system.[21] Under circumstances where bacteria does disseminate into the bloodstream, IgG, which is a powerful opsonin and activator of the complement system, can facilitate clearance of the bacteria.[21,24] The LCP system also features a lysine (K) moiety (or moieties) from which different vaccine components can be attached.[25] These components can be placed and rearranged at the α-amino, α-carboxyl, and ε-amino functional groups of the central lysine. This allows for the design of LCP systems with different molecular geometries but containing the same epitopes.

Conventionally, a typical GAS LCP vaccine consists of C12 (2-amino-D,L-dodecanoic acid) LAAs attached on the α-carboxyl side-chain of the lysine core via glycine spacers, and peptide epitopes (usually B-cell epitopes) attached to the lysine amine moieties.[26] In recent designs of vaccine candidates against GAS, a T-helper (Th) epitope (P25) was included on the N-terminus to confer immunity in heterologous populations, two copies of serine (S) were included as spacer, and the C12 LAA was replaced with the more lipophilic C16 LAA (2-amino-D,L-hexadecanoic acid).[26,27] The newly designed LCP was able to stimulate IgG antibody production upon intranasal delivery.[17,28] These alterations suggest that structural changes to an LCP have the potential to affect its immunological properties.

Herein, LCP vaccine candidates (Fig. 1) were designed to incorporate the same J14 B-cell epitope and P25 Th-epitope, but to differ in lipopeptide structure (LAA side-chain length) or spatial orientation. Following LCP synthesis and self-assembly into nanoparticles, immunological evaluation was performed upon intranasal administration in mice. Both spatial orientation of epitopes and lipophilicity of LAAs were found to influence humoral immune responses generated by LCP **1**–**4**.

2. Materials

Protected L-amino acids were purchased from Mimotopes (Melbourne, Australia) or Novabiochem (Laufelfingen, Switzerland). pMBHA resin was obtained from Peptide International Inc. (Kentucky, USA). Trifluoroacetic acid (TFA) was purchased from Merck (Kilsyth, Australia). 1-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was obtained from Mimotopes. HPLC grade acetonitrile (MeCN) and *N,N*-dimethylformamide (DMF) were purchased from Ajax Finechem (Sydney, Australia). All other reagents were obtained from Sigma–Aldrich (Castle Hill, NSW, Australia). Microwave-assisted Fmoc-SPPS was performed on a CEM Discovery reactor (CME Corporation, Matthews, NC, USA). Microwave-assisted Boc-SPPS was carried out using a CEM Discovery automated peptide synthesiser. HF cleavage was achieved with an AKel-F HF apparatus (Peptide Institute, Osaka, Japan). ESI-MS was performed on a Perkin-Elmer-Sciex API3000 with Analyst 1.4 software (Applied Biosystems/MDS Sciex, Toronto, Canada). Analytical RP-HPLC was performed on an Agilent instrument. Separation was achieved by running gradient mode of solvent B (MeCN/H_2O/TFA; 90:10:0.1) over solvent A (H_2O/TFA; 100:0.1) on a Vydac analytical C4-column (214TP54; 10 μm, 4.6 × 250 mm) or a Vydac analytical C18-column (218TP54; 10 μm, 4.6 × 250 mm). Purification was performed on a preparative RP-HPLC using a Waters Delta 600 system with a 10.0 ml/min flow rate. Compounds were detected at 230 nm and separation was achieved with solvent B and solvent A on a C4-column (214TP1022; 10 mm, 22 × 250 mm). DLS (dynamic light scattering) measurements were taken on a Nanosizer instrument (Zetasizer Nano Series ZS, Malvern Instruments, Worcestershire, UK) using the Zetasizer 6.2 software. Particle-imaging was achieved with a JEM-1010 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

3. Methods

3.1. Synthesis of LAAs with Dde-protective group (Dde-C16 and Dde-C20)

C16 (2-amino-D,L-hexadecanoic acid) and C20 (2-amino-D,L-eicosanoic acid) were synthesised following similar methods

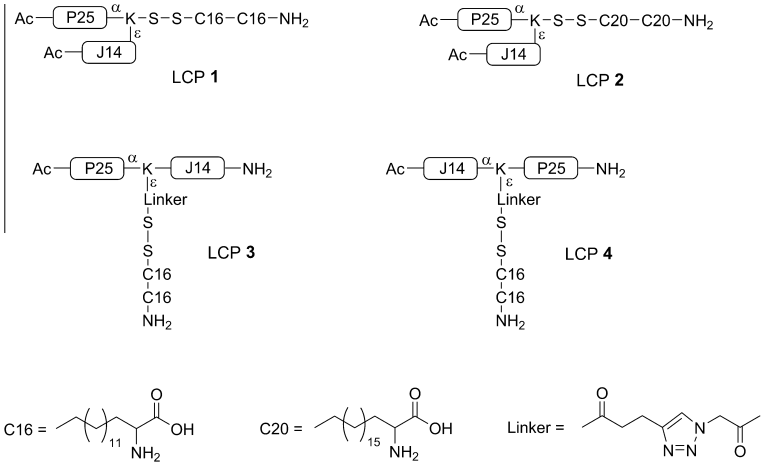

Figure 1. Structures of synthetic LCP **1**–**4**. The LCPs incorporated a GAS B-cell epitope (J14: KQAEDKVKASREAKKQVEKALEQLEDKVK), a universal Th-epitope (P25: KLIPNASLIENCTKAEL), and LAAs with differing side-chain length (C16 or C20). Ac = acetyl group; S = serine; K = lysine.