

## The importance of epitope and lipid orientation in the design of synthetic self-adjvanting group A streptococcal (GAS) vaccine

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### Introduction

“Strep throat” is mostly caused by a *Streptococcus pyogenes*, a β-haemolytic bacterium commonly referred to as group A streptococcus (GAS). GAS is also responsible for a number of clinical conditions ranging from upper respiratory tract and skin infections to invasive diseases. While these infections are often easily treated, delayed or inadequate treatment of GAS infections can lead to immune-mediated post-streptococcal sequelae known as rheumatic fever (RF) and rheumatic heart disease (RHD).<sup>1</sup> GAS-associated diseases are responsible for approximately 517,000 deaths per annum.<sup>2</sup>

Our vaccine strategy is to raise protective antibodies against J14, a 29-mer chimeric peptide derived from the conserved C-terminus of the GAS M-protein. We are developing novel synthetic self-adjvanting vaccine constructs (Fig. 1), composed of (i) a universal helper T cell epitope (P25), (ii) a target GAS B-cell epitope (J14), and (iii) a lipid moiety targeting Toll-like receptor 2 (TLR2). The T-helper cell epitope (P25) was used to generate the proliferation of T cells eliminating the use of protein carriers. The lipid moiety was designed using lipoamino acids to impart a self-adjvanting activity to the short peptide J14.

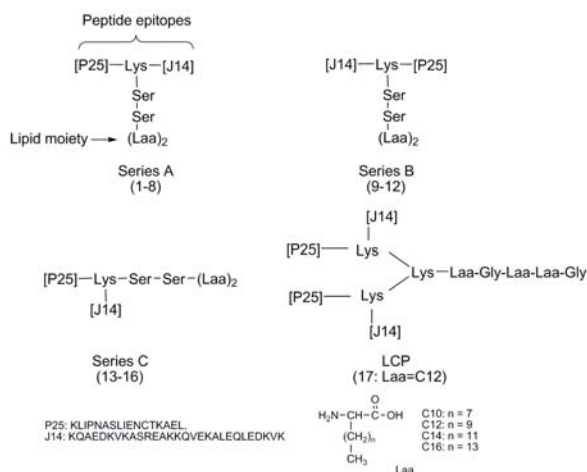


Fig. 1. Structure of the GAS vaccine constructs

### Results and Discussion

Lipopeptide vaccine candidates were assembled by manual SPPS using Boc-chemistry in situ neutralization/HBTU method, purified by preparative

HPLC and characterized by ES-MS spectrometry and SDS gel electrophoresis.

Immunological evaluation was performed in female BALB/c (H-2<sup>d</sup>) mice (5 per group) subcutaneously (Fig. 2) or intranasally (Fig. 3), without an additional adjuvant. The positive control group was administered J14-diphtheria toxoid conjugate (J14-DT) or J14 emulsified with Freund's complete adjuvant (CFA), while the negative control groups were administered CFA or PBS. Mice were immunized on day (0, 21, 31 and 41 for the *s. c.* experiment) and on day (0, 21 and 41 for the intranasal experiment) with 30 μg of immunogens dissolved in phosphate-buffered saline (PBS). Sera were collected prior to each boost, and nine days after the final boost, to assess the levels of J14-specific serum IgG elicited using an enzyme-linked immunosorbent assay (ELISA). All protocols were approved by the Bancroft Centre Research Ethics Committee and were carried out according to Australian National Health and Medical Research guidelines.

The best vaccines featured a C-terminal lipid moiety, conjugated through a lysine residue to P25 at the N-terminus, and J14 on the lysine side-chain. A preliminary investigation has shown the ability of our new constructs to activate Toll-like receptor 2 (Fig. 4).

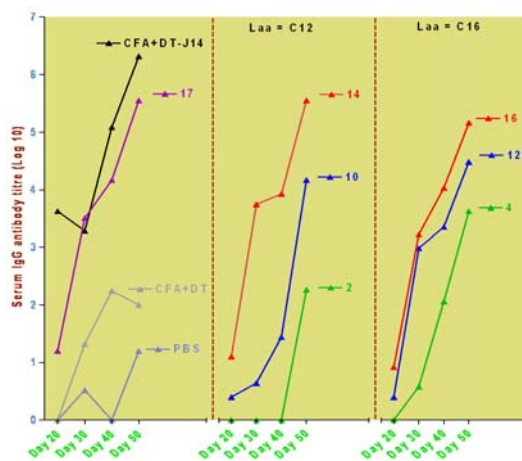


Fig. 2. Time-course for J14-specific serum IgG antibody titers (log<sub>10</sub>) elicited in response to subcutaneous immunization of BALB/c (H-2<sup>d</sup>) mice at the tail base with lipopeptides, as determined by ELISA.

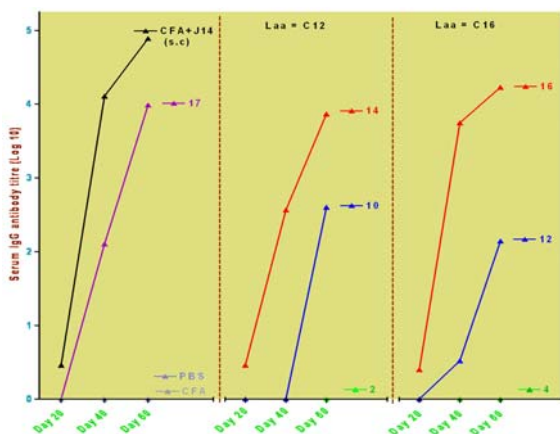


Fig. 3. Time-course for J14-specific serum IgG antibody titers (log10) elicited in response to intranasal immunization of BALB/c (H-2d) mice with lipopeptides, as determined by ELISA.

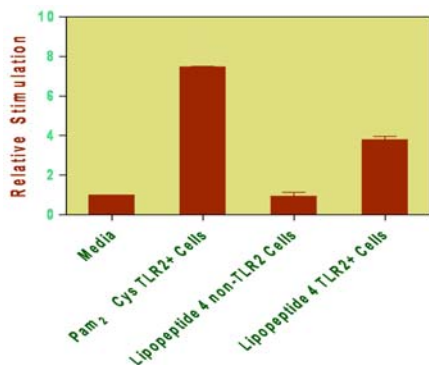


Fig. 4. The ability of the lipid to signal through TLR. Lipopeptide 4 was administered to 293HEK cells stably expressing human TLR2, and to blank 293HEK cells, and then harvested into a cell lysis buffer. The results were shown relative to TLR2-expressing cells treated with media.

### Acknowledgments

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### References

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