Introduction

Streptococcus pyogenes, also known as Group A Streptococcus (GAS) is a major human pathogen that is the cause of diseases such as pharyngitis, tonsillitis, necrotising fasciitis, rheumatic fever and toxic shock syndrome. Rates of acute rheumatic fever in New Zealand have failed to decrease since the 80s and remain one of the highest reported in developed countries.

GAS pili are hair-like appendages extending from the cell surface. They are generally involved in cell adhesion, and are required for colonization during infection. The genes that encode pilus-associated proteins are located within a variable genomic island known as the FCT (fibronectin-collagen-T-antigen) region, of which there are currently nine known variants (Fig. 1).

A previous study from our group has shown that GASPEL (GAS Lactic acid bacteria) has become a popular vaccine vehicle due to its safety and natural adjuvancy. A previous study from our group has shown that GASPEL (GAS Lactic acid bacteria) has become a popular vaccine vehicle due to its safety and natural adjuvancy. GASPEL – a novel mucosal vaccine against Group A Streptococcus

Figure 1: Schematic representation of the nine known FCT genetic regions of GAS (adapted from Falugi et al). The FCT-7 genotype is a derivative of FCT-4, where the minor pilin 1 is absent. Another derivative of the FCT-4 genotype, in which the transcriptional regulator mna replaces rofA, is designated FCT-8.

Figure 2: Schematic representation of pilus assembly on the cell surface. The major pilin forms the core of the pilus structure. Minor pilin 1 typically sits at the tip, while minor pilin 2 typically sits at the base. Sortases catalyse the covalent linkage of the protein subunits. Pili of various lengths are formed as differing number of major pilin subunits are incorporated.

Figure 3: A representative Western blot where antibodies against the major pilin of M28 were used for detection. The positive control (Lane 1) shows purified recombinant M28 major pilin. Negative control (Lane 2) shows cell wall extract from L. lactis carrying empty vector. High molecular weight laddering of typical pilus expression is observed in cell wall extract from L. lactis carrying vector containing the M28 pilus operon (Lane 3).

Figure 4: Cross-reactivity of PilM18 polyclonal antibodies to the major pilins from GAS strains with various FCT regions.

Figure 5: Cross-reactivity of PilM28 polyclonal antibodies to the major pilins from GAS strains with various FCT regions.

Figure 6: Analysis of PilM18 polyclonal antibodies with minor pilin 1 from selected GAS strains.

Figure 7: Analysis of PilM28 polyclonal antibodies with minor pilin 1 from selected GAS strains.

Aim

This pilot study aims to test two recombinant Lactococcus lactis strains, each carries a different GAS pili type, PilM18 (FCT-3) and PilM28 (FCT-4), respectively, as live vaccines in rabbits. Cross-reactivity to the major and minor pilin subunits of a panel of GAS strains will be analyzed in post-immunization antibodies.

Results

The serum from rabbits vaccinated with PilM18 and PilM28 were tested for cross-reactivity with the major pilin and minor pilin 1 from various GAS strains by ELISA.

Conclusion and Future Work

The rabbit serum immunized with PilM28 (FCT-4) and PilM18 (FCT-3) showed cross-reactivity to the major pilin and/or minor pilin 1 of various GAS strains. The remaining pilus types in GAS (FCT-1, FCT-2, FCT-5 and FCT-6) will also be studied to evaluate the efficacy of GASPEL against a range of clinically important GAS strains. The vaccine will be tested for neutralizing and protective antibodies against GAS in rabbits and mice.

References