Methods

Peanut agglutinin Overlapping

ogcBE

△ogcI

Introduction

Protein glycosylation is a post-translational modification widespread in prokaryotes. Glycans are attached to the amide nitrogen of asparagine residues (N-glycosylation) or to the hydroxyl oxygen of serine/threonine residues (O-glycosylation). Bacterial glycoproteins may play important roles in pathogenicity, motility, and protection against gut proteases, promoting robustness of the bacterium in the host environment.

*B. cenocepacia* has a general O-glycosylation system that is O-Tase dependent and it can glycosylate at least 23 proteins. We have recently identified the *Burkholderia* O-glycosylation gene cluster (ogc) (Fig. 1) responsible for the step-wise assembly and membrane translocation of a lipid-linked trisaccharide glycan, which was conserved among all species of *Burkholderia* (Fig. 2). We also found that sera from patients infected with various *Burkholderia* species have antibodies that recognize the glycan component of a model glycoprotein (Fig. 3). In this work, we describe the construction of two classes of glycoengineered putative anti-*Burkholderia* vaccines.

Recombinant glycoprotein-based vaccine: covalent coupling of trisaccharide to a carrier protein to generate a glycoconjugate.

E. coli LPS-display vaccine: to develop a strain expressing a lipid A-core oligosaccharide substituted with *B. cenocepacia* trisaccharide glycan in a fashion resembling O-antigen.

Results

Figure 1. Analysis of recombinant proteins expressed in *Burkholderia* parental strain and glycotransference-deficient mutant strain. The presence of the glycan was observed in recombinant proteins expressed in *B. cenocepacia* parental strain, but not in proteins expressed by the glycotransference-deficient mutant strain.

Silver-stained gel

Lectin blot (PNA)

Figure 2. Analysis of LPS extractions from various *E. coli* strains with/without constructed plasmid expressing ogc cluster by silver-stained gel and fluorescent lectin blot. An additional moiety consistent with the *B. cenocepacia* trisaccharide glycan was detected in a ΔwecA mutant, but not in a ΔHaaR mutant.

Conclusions

We successfully produced two prototypes of putative anti-*Burkholderia* vaccines: a recombinant glycoprotein-based vaccine and an *E. coli* LPS-display vaccine. Our results demonstrate that the O-glycosylation pathway can be manipulated for the construction of potential anti-*Burkholderia* vaccines.