Potential glycoengineered anti-*Burkholderia* vaccines by exploiting the bacterial O-glycosylation machinery

Guanbo Wang, Lena Glaser, Yasmine Fathy Mohamed, Rebecca Ingram, and Miguel A. Valvano
Welcome-Wolfson Institute for Experimental Medicine, Queen’s university Belfast

**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 OTase 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.

**Methods**

**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**

More than 90% sera from individuals with *Burkholderia* infections had antibodies that specifically recognize a glycosylated protein purified from *Burkholderia cenocepacia* (clear circles), but not the unglycosylated protein purified from the glycosylation defective ΔwecA mutant (filled magenta circles). The dotted line denote the cut-off value below which the results correspond to negative ELISA values.

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.

**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.