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Structures and immunochemical properties of endotoxin derived oligosaccharides and neoglycoconjugates as vaccine components

Abstract

Glycoconjugate vaccines combine immunogenic properties of saccharide and protein antigens. The protein component improves immunogenicity of carbohydrate. Moreover, conjugates are well characterized, reducing concerns about vaccine safety. This study focused on assessing immunogenic properties of core oligosaccharides originating from Gram-negative bacteria and toxoids from Gram-positive bacteria. The synthetised glycoconjugates were designed to be safe and efficient immunogens towards broad spectrum of bacterial pathogens. Analytical methods engaged in structural characterisation of carbohydrate and protein antigens, as well as conjugates, involved mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

Core oligosaccharides (R1 and R1Z) derived from *Escherichia coli* type R1 lipooligosaccharide (LOS) were isolated using hydrophilic interaction liquid chromatography (HILIC) on ZIC®-HILIC column. The column was coupled to mass spectrometer with electrospray ion source, allowing for on-line analysis of eluent, and separation of highly homologous saccharides. The first protein carrier (TBd) was C-terminal fragment of receptor binding domain of *Clostridium difficile* toxin B, which exerts no cytotoxic activity. The TBd protein was selected and designed, as a potential component of a vaccine against *C. difficile* and protein carrier in glycoconjugate. The second protein antigen was tetanus toxoid (TTd), commonly used as protein carrier. The R1 and R1Z oligosaccharides were conjugated to protein in order to prepare glycoconjugates, which were characterized using immunoblotting or MS techniques and further used *in vivo* for rabbit immunisation.

Post immunisation sera, with strongest response to R1 type LOS, were tested for cross-reactivity with LOSs and lipopolysaccharides (LPSs) from 33 selected Gram-negative bacteria. Antibodies cross-reacted with LOS and LPS bearing core oligosaccharides homologous or related to type R1. Moreover, anti-R1Z serum antibodies reacted with LPSs from *Bordetella pertussis* 186 and *Klebsiella pneumoniae* O3 and O5. Binding of serum antibodies to LPS and LOS present on intact rough and smooth *E. coli* R1, O18 and O39,

Klebsiella pneumoniae O3 and O5 and *Bordetella pertussis* 186 strains was detected in flow cytometry. Additionally, sera had bactericidal activity towards above mentioned strains.

Saccharide epitopes responsible for interaction with immunoglobulins G, isolated from serum, were analysed for R1, R1Z and *B. pertussis* 186 oligosaccharides, using STD NMR. For *E. coli* oligosaccharides, mainly outer core residues were responsible for binding, with no differences noticed for the two glycoforms. Analysis of *B. pertussis* antigen reveals that terminal trisaccharide is responsible for strong interaction with antibodies.