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Structural Studies on Lipopolysaccharides from *Haemophilus* Species

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ABSTRACT

Background: Carbohydrates are indispensable mediators for a variety of cellular interactions. In biological systems carbohydrates are usually linked to a carrier as e.g. proteins or lipids. One example is the molecule lipopolysaccharide (LPS). LPS is one of the major outer membrane constituents found in Gram-negative bacteria and they play key roles in the biology of these organisms. Notably, they have been found to be important virulence factors in pathogenic species.

Aims: In this thesis the biosynthesis and the molecular structures of LPS expressed by *Haemophilus influenzae* and *Haemophilus parainfluenzae* were investigated. These two bacteria colonize the human nasopharynx. *H. influenzae* with capsule type b is involved in invasive diseases such as meningitidis and epiglottis while non-encapsulated *H. influenzae* (non-typeable, NTHi) often cause otitis media, and acute and chronic lower respiratory tract infections in infants. Though, closely related *H. parainfluenzae* is a commensal.

Materials and Methods: Structural elucidation of LPS involves initial de-lipidation to obtain water-soluble material that is suitable for subsequent analyses by chemical, nuclear magnetic resonance (NMR) and mass spectrometric (MS) methods.

Results: The function of the gene *lic2B* in *H. influenzae* type b strain Eagan was investigated. The LPS expressed by the mutant strain Eagan*lic2B*lic2C+ was analyzed and found to encode for a glucosyltransferase responsible for the addition of β-D-Glc*p* to O-4 of α-D-Glc*p*-(1→ elongating from the middle inner core heptose. Further, the LPS structures of NTHi strains 1247 and 1008 were determined. NTHi strain 1247 expressed globotetraose elongating from the phosphocholine bearing GlcI, [β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→4)-[*P*Cho→6]-β-D-GlcI*p*-(1→], or truncated versions thereof. Globotetraose was also found to elongate from the distal heptose in NTHi 1247. The *lpsA* mutant of strain 1247 allowed the identification of a novel disialyllactose epitope, [α-Neu5Ac-(2→8)-α-Neu5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→], from the proximal inner core heptose. Alternatively, a globotetraose was found to elongate from GlcI. NTHi strain 1008 showed almost identical glycoforms to NTHi strain 1247 but lacked terminal *N*-acetyl galactoseamine.
All of the three investigated *H. parainfluenzae* strains were shown to express the same lipid A and inner core as NTHi. *H. parainfluenzae* genome strain T3T1 and strain 22 were shown to express rough-type LPS having novel outer core structures elongating from GlcI that were [α–Neu5,9Ac$_2$-(2→6)-β-D-Gal*p*NAc-(1→4)-β-D-Gal*p*-(1→3)-β-D-Fuc*p*NAc4N-(1→] in strain T3T1 and [Neu5Ac-(2→6)-α-D-Gal*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Fuc*p*NAc4N-(1→] in strain 22. *H. parainfluenzae* strain 13 expressed an O-repeating chain with the structure [→6)-[Ac→3]-β-D-Gal*f*-(1→3)-[*P*Etn→6]-β-D-Glc*p*NAc-(1→].

Conclusion: It was showed that the *H. parainfluenzae* LPS investigated here lacks all virulence determining LPS attributes expressed by *H. influenzae* such as phosphocholine and phase variable expression of outer core structures. This may provide further insight into the factors relating to commensal vs. pathogenic behavior inside the host.

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