Studies of Carbohydrate-Binding Proteins from Enterotoxigenic *Escherichia coli*

AKADEMISK AVHANDLING

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av
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Fakultetsopponent: Professor Monica Palcic, Carlsberg Laboratory, Köpenhamn, Danmark

Avhandlingen baseras på följande delarbeten:

I. The major subunit, CfaB, of colonization factor antigen I from enterotoxigenic *Escherichia coli* is a glycosphingolipid binding protein. Lena Jansson, Joshua Tobias, Michael Lebens, Ann-Mari Svennerholm, and Susann Teneberg. 2006. Infect. Immun.74, 3488-3497.

II. Sulfatide recognition by colonization factor antigen CS6 from enterotoxigenic *Escherichia coli*. Lena Jansson, Joshua Tobias, Catharina Jarefjäll, Michael Lebens, Ann-Mari Svennerholm, and Susann Teneberg. 2009. PLoS ONE 4: e4487.

III. No direct binding of the heat-labile enterotoxin of *Escherichia coli* to lipopolysaccharides. Lena Jansson, Jonas Ångström, Michael Lebens, and Susann Teneberg.
Submitted manuscript

IV. Carbohydrate binding specificities of the cholera toxin-like B-subunit from *Citrobacter freundii*. Lena Jansson, Michael Lebens, and Susann Teneberg.
Manuscript

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Studies of Carbohydrate-Binding Proteins from Enterotoxigenic *Escherichia coli*

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Knowledge about the carbohydrate binding specificity of pathogens can give an increased understanding of their virulence strategies, and is also of importance for the development of anti-adhesive therapies and vaccines. Enterotoxigenic *Escherichia coli* (ETEC) is a well-known causative agent of diarrheal disease. The first step in the pathogenesis of ETEC infections is adhesion of the bacterium to the small intestinal epithelium, mediated by colonization factors (CFs). Two of the most common CFs are CFA/I and CS6. CFA/I is a fimbriae with a major subunit, CfaB, and a minor subunit, CfaE. CS6 is non-fimbrial and composed of two major subunits, CssA and CssB.

The potential carbohydrate recognition by CFA/I was investigated by binding CFA/I-fimbriated bacteria and purified CFA/I fimbriae to variant glycosphingolipids on thin-layer chromatograms. Both with CFA/I bacteria and purified fimbriae binding to a number of non-acid glycosphingolipids was obtained. These compounds were also recognized by CFA/I in the absence of CfaE, demonstrating that the glycosphingolipid-binding of CFA/I resides within CfaB. Using CS6-expressing *E. coli*, and purified CS6 protein, a highly specific binding to sulfatide (SO_3-3Galβ1Cer) was obtained. The binding of the CS6 protein and CS6-bacteria to sulfatide was inhibited by dextran sulfate, but not by dextran, heparin, galactose 4-sulfate or galactose 6-sulfate, suggesting that sulfate in position 3 is of importance for binding of CS6. When using recombinantly expressed and purified CssA and CssB, sulfatide binding was obtained with CssB, demonstrating that this subunit carries the glycolipid binding capacity. CS6-binding sulfatide is present in the small intestine of species susceptible to CS6-mediated infection, as humans and rabbits, but lacking in species not affected by CS6 ETEC, as mice. The ability of CS6-expressing ETEC to adhere to sulfatide in target small intestinal epithelium may thus contribute to virulence.

The heat-labile enterotoxin of ETEC is an AB_5 toxin, with a pentamer of receptor-binding B-subunits. B-subunits from porcine and human isolates of ETEC (pLTB and hLTB, respectively) and the B-subunit of cholera toxin (CTB) are highly similar, and all of them bind to the GM1 ganglioside. pLTB also binds neolacto sequences and hLTB blood group A type 2 sequences. A B-subunit (CFXB), with 73% sequence identity to CTB, is found in *Citrobacter freundii* and some ETEC strains. Binding studies showed that CFXB binds with high affinity to GM1, and to both neolacto and blood group A sequences. The blood group antigen A binding site of hLTB has been proposed to be involved in binding of hLTB to lipopolysaccharides (LPS). Our study aimed at defining the relationship between the blood group A/B binding site and the LPS binding site. However, no binding of the B-subunits to *E. coli* LPS was obtained, and incubation with LPS did not affect the binding of hLTB to blood group A-determinants, indicating that the blood group A binding site is not involved in LPS binding. Incubation with blood group A saccharide inhibited the binding of hLTB to GM1, and *vice versa*, suggesting that a concurrent occupancy of the two binding sites does not occur.

Key words: Carbohydrate recognition/ETEC colonization factors/CFA/I fimbriae/CS6 protein/heat-labile enterotoxin

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