

# Studies of glycosphingolipids in infection, immunity and differentiation

## Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien vid Göteborgs universitet kommer att offentligens försvaras i hörsal Ragnar Sandberg, Medicinargatan 7A, Göteborg, fredagen den 8 maj 2015, kl. 9.00

av

**John Benktander**

Fakultetsopponent: **Prof. Dr. Johannes Müthing**  
Institute for Hygiene, University of Münster, Germany

Avhandlingen baseras på följande arbeten:

- I. Benktander J, Ångström J, Breimer ME, Teneberg S. (2012) **Re-definition of the carbohydrate binding specificity of *Helicobacter pylori* BabA adhesin.** *J Biol Chem.* 287, 31712-24.
- II. Benktander J, Ångström J, Karlsson H, Teymournejad O, Lindén S, Lebens M, Teneberg S. (2013) **The repertoire of glycosphingolipids recognized by *Vibrio cholerae*.** *PLoS One.* 8, e53999.
- III. Barone A, Benktander J, Ångström J, Aspegren A, Björquist P, Teneberg S, Breimer ME. (2013) **Structural complexity of non-acid glycosphingolipids in human embryonic stem cells grown under feeder-free conditions.** *J Biol Chem.* 288, 10035-50.
- IV. Westman JS, Benktander J, Storry JR, Peyrard T, Hult AK, Hellberg A, Teneberg S, Olsson ML. **Genetic basis of PX2, a recently acknowledged glycolipid blood group antigen.** Submitted.



UNIVERSITY OF GOTHENBURG

**Göteborg 2015**

# Studies of glycosphingolipids in infection, immunity and differentiation

**John Benktander**, Department of Medical Biochemistry, Institute of Biomedicine, University of Gothenburg, Göteborg, Sweden 2015.

Cell surface glycoconjugates play a role in many biological processes such as responses to microbial infections, cell-cell interactions, differentiation, and inflammatory responses. The present work is focused on structural characterization of glycosphingolipids with potential roles in adhesion of *Helicobacter pylori* and *Vibrio cholerae*, differentiation of human pluripotent stem cells, and as blood group determinants.

In the first study, the structural binding requirements of *Helicobacter pylori* BabA adhesin revealed a different carbohydrate binding potential than previously defined. Adhesion of *H. pylori* generalist, specialist and BabA deletion mutant strains were examined using mixtures of glycosphingolipids. An unexpected binding by specialist and generalist *H. pylori* to the hexaosylceramide region of porcine intestinal non-acid glycosphingolipids was found. After isolation and characterization by mass spectrometry and proton NMR, the binding-active glycosphingolipid was determined as Globo H hexaosylceramide (H type 4). Further binding studies demonstrated that the generalist strain, but not the specialist strain, also recognized Globo A heptaosylceramide (A type 4). Non-secretors have an increased risk of peptic ulcer disease although they express little or no H type 1 sequences, and thus no Le<sup>b</sup>. However, these individuals have a functional FUT1 enzyme that may produce the Globo H sequence, suggesting that Globo H hexaosylceramide might have a role in *H. pylori* adhesion to the gastric epithelium of non-secretor individuals.

In the second study the carbohydrate binding potential of *Vibrio cholerae* was investigated. Binding-active glycosphingolipids, detected by the thin-layer chromatogram binding assay, were isolated and characterized by antibody binding, mass spectrometry and proton NMR. Thereby, three different binding modes were identified; the first was complex glycosphingo-lipids with GlcNAc $\beta$ 3Gal $\beta$ 3/4GlcNAc sequence, the second glycosphingolipids with terminal Gal $\alpha$ 3Gal $\alpha$ 3Gal sequence, and the third lactosylceramide and related glycosphingolipids.

*V. cholerae* with non-functional chitin binding protein GbpA bound to glycosphingolipids in the same manner as the wild type bacteria, demonstrating that the GbpA is not involved in glycosphingolipid recognition.

In the third study the non-acid glycosphingolipids of human embryonic stem cells were structurally characterized. Immunohistochemistry, mass spectrometry and proton NMR demonstrated the presence of type 2 core chain glycosphingolipids (*neolactotetraosyl*-ceramide, H type 2 pentaosylceramide, Le<sup>x</sup> pentaosylceramide, and Le<sup>y</sup> hexaosylceramide), and blood group A type 1 hexaosylceramide, along with the previously characterized glycosphingolipids with type 1 and type 4 core chains. Thus, the glycosphingolipid diversity of human embryonic stem cells is more complex than previously appreciated.

The PX2 antigen is assumed to belong to the GLOB blood group system and has until further notice been assigned to that blood group. However the enzymatic machinery involved in PX2 synthesis has not been determined. In the fourth study, glycosphingolipids isolated from blood group AP<sub>1</sub><sup>k</sup> erythrocytes, App erythrocytes and *B3GALNT1* transfected MEG-01 cells were characterized by antibody binding and mass spectrometry. The *B3GALNT1* transfected MEG-01 cells had an increased expression of PX2. No P antigen or PX2 were found in the AP<sub>1</sub><sup>k</sup> erythrocytes, while the App erythrocytes expressed PX2, but no P1 and P antigens. The conclusion from these experiments is that the P synthase also is responsible for synthesis of the PX2 antigen.

**Keywords:** Glycosphingolipids, mass spectrometry, *Helicobacter pylori* BabA, *Vibrio cholerae*, human embryonic stem cells, glycosyltransferase, PX2.

ISBN: 978-91-628-9321-7