

# **Norovirus, causative agent of winter vomiting disease, exploits several histo-blood group glycans for adhesion**

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Norovirus is recognized as the major cause of outbreaks of gastroenteritis world-wide, yet no vaccines or drugs are available for prevention or treatment of the virus infection. Challenge studies and binding studies using virus-like particles (VLPs) have suggested susceptibility to norovirus infection to be associated with secretor status.

This thesis supports this idea by demonstrating that among 105 Swedish blood donors, non-secretors had significantly lower plasma titers of norovirus genogroup (G) II.4 specific IgG antibodies than secretors ( $p < 0.0001$ ). However, some non-secretors had high antibody titers, indicating that secretor independent strains also exist.

In lack of *in vitro* cultivation methods, VLPs were used to characterize the glycan binding characteristics of different norovirus strains. VLPs from the Chron1 (GII.3) and the Dijon (GII.4) strain recognized saliva samples from secretors, but not from non-secretors. Using neoglycoproteins, the two VLPs were shown to recognize sialyl Lewis x and the structural analogues sialyl diLewis x and sialylated type 2 in addition to secretor gene dependent glycans. In contrast, VLPs from the Norwalk (GI.1) strain only recognized secretor gene dependent glycans. In inhibition experiments, the sialyl Lewis x conjugate could completely block binding of the Chron1 and Dijon VLP to saliva samples.

In search for receptor glycoconjugates, human norovirus VLPs were for the first time demonstrated to bind to glycosphingolipids. Using a chromatogram binding assay, radiolabeled Norwalk VLPs were shown to recognize both type 1 and type 2 chain glycosphingolipids terminated with blood group A and H, but not B epitopes. Quartz crystal microbalance with dissipation (QCM-D) monitoring was used to characterize VLP binding to glycosphingolipids in supported lipid bilayers. The Norwalk and the Dijon VLP bound to bilayers containing H type 1, but not to those containing Lewis a glycosphingolipids. In support of multivalency, both VLPs showed a threshold concentration of H type 1 below which no binding was observed.

To conclude, this thesis describes a wide variety of histo-blood group glycoconjugates recognized by human noroviruses, suggesting novel approaches for design of glycomimetics for norovirus anti-adhesion therapy.

**Keywords:** norovirus, glycobiology, virus-like particle, *FUT2*, ABO(H) histo-blood group antigen, sialyl Lewis x, neoglycoprotein, glycosphingolipid, QCM-D, supported lipid bilayer

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- I. Larsson, M. M., **Rydell, G. E. P.**, Grahn, A., Rodriguez-Diaz, J., Åkerlind, B., Hutson, A. M., Estes, M. K., Larson, G. and Svensson, L., (2006) Antibody prevalence and titer to norovirus (genogroup II) correlate with secretor (*FUT2*) but not with ABO phenotype or Lewis (*FUT3*) genotype, *The Journal of Infectious Diseases*, 194(10), 1422-7
- II. **Rydell, G. E.**, Nilsson, J., Rodriguez-Diaz, J., Ruvoën-Clouet N., Svensson, L., Le Pendu, J. and Larson, G., (2009) Human noroviruses recognize sialyl Lewis x neoglycoprotein, *Glycobiology* 19(3), 309-20
- III. Nilsson, J., **Rydell, G. E.**, Le Pendu, J. and Larson, G., (2009) Norwalk virus-like particles bind specifically to A, H and difucosylated Lewis but not to B histo-blood group active glycosphingolipids, *Glycoconjugate Journal*, Apr 23 DOI 10.1007/s10719-009-9237-x
- IV. **Rydell, G. E.**, Dahlin, A. B., Höök, F. and Larson, G., QCM-D studies of human norovirus VLPs binding to glycosphingolipids in supported lipid bilayers reveal strain specific characteristics, *Manuscript*



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