

The MUC2 mucin -A network in the intestinal protective mucus

AKADEMISK AVHANDLING

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av

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This thesis is based on the following papers:

- I. **Johansson, M. E. V.**, Godl, K., Lidell, M. E., Morgelin, M., Karlsson, H., Olson, F. J., Gum, J. R. Jr., Kim, Y. S., and Hansson, G. C. (2002). The N terminus of the MUC2 mucin forms trimers that are held together within a trypsin-resistant core fragment. *J. Biol. Chem.* 277, 47248-56.
- II. **Johansson, M. E.V.**, Phillipson, M., Petersson, J., Velchich, A., Holm, L., and Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl. Acad. Sci. U S A.* 105, 15064-9.
- III. **Johansson, M. E. V.**, Thomsson, K. A., and Hansson, G. C. (2009). Proteomic analysis of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the Fcgbp protein. *J. Proteome Res.*, *in press*.
- IV. **Johansson, M. E. V.**, and Hansson, G. C. (2009). Mucus turnover in the intestine measured by *in vivo* labeling of glycoproteins. *Manuscript*.
- V. **Johansson, M. E. V.**, Gustafsson, J., Petersson, J., Holm, L., Sjövall, H., and Hansson, G. C. (2009). The colitis inducing agent Dextran sulfate disrupts the colon inner mucus gel and allows bacteria to penetrate before inflammation is observed. *Submitted manuscript*.



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ABSTRACT

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The intestine is covered by mucus that is the first line of defence of the epithelium. The main structural component of the intestinal mucus is the MUC2 mucin. This is a large glycoprotein with two long and heavily *O*-glycosylated mucin domains. Our studies of the biosynthesis have revealed that MUC2 forms large disulphide linked networks starting with C-C terminal dimers. In the late secretory pathway the N-terminal of MUC2 forms trimers within a core fragment resistant to trypsin cleavage. The MUC2 assembly creates an enormous network with an ability to resist protease degradation. It is important that the mucus is resistant to the intestinal digestive enzymes.

In colon, the real challenge is to wield the large number of bacteria in the normal flora. Immune tolerance has been studied intensely, but the contribution of the mucus in the protective function has been neglected largely due to the technical difficulties to work with the large mucin glycoproteins. The mucus in colon is made up of two mucus layers. In mouse the inner mucus layer is 50 μm thick and firmly attached to the epithelium. This is a compact, insoluble and stratified mucus layer with a high Muc2 concentration. The firm layer is converted to a 100 μm , soluble, loose overlaying mucus layer that is expanded in volume by proteolysis. The mucus turnover is fast and in colon the luminal mucus layers are renewed in hours. The composition of the mucus was investigated by proteomics and was found to be similar in the two mucus layers, indicating a common source. One of the components identified, Fc gamma binding protein (Fcgbp) was shown by purification of the mucus in guanidinium chloride to be covalently attached to Muc2. The binding may be mediated by potential autocatalytic cleavage sites that generate new reactive C-termini in Fcgbp. The disulphide stabilized Fcgbp could thus be a cross-linker of the Muc2 network.

Bacteria in colon were detected in the outer loose mucus layer by *in-situ* hybridization using a 16S rRNA general bacterial probe. This mucus is likely to be a good habitat for bacteria providing binding sites and energy. The inner compact firm mucus is impervious to bacteria, making it a protective barrier for the enormous bacterial load. The mucus is through this mechanism a part of the innate immunity to keep the homeostasis in colon.

The protective function of mucus argues for that defects in the mucus can be a cause of inflammation. In fact, mice with the Muc2 gene disrupted do not produce mucus and develop spontaneous colitis. In these animals the epithelium is in direct contact with the colonic flora, bacteria enter deep into the normally sterile crypts and penetrate the epithelial cells. An overt immune reaction to the bacteria is an obvious cause of the inflammation. In wild type mice dextran sulphate (DSS), a highly sulphated glucose polymer, is used to induce colitis and is the most common UC model. DSS exposure resulted in alterations in the mucus long before any signs of inflammation were observed. The inner mucus allowed bacteria to penetrate as early as after 4 h of exposure, with a massive bacterial penetration into the inner mucus after 12 h. The mechanisms behind this colitis model were not known until now when our observations suggest that a defect mucus layer is likely to have triggered the inflammation. The importance of the inner mucus for epithelial protection argues for defective mucus as a possible cause of ulcerative colitis.

Keywords: mucus, Muc2, trimer, proteomics, intestine, colitis, DSS