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S10.5

Binding of *Clostridium difficile* Toxin A to Glycosphingolipids; Identification of a Novel Binding-Active Carbohydrate Sequence Present in Human Tissue

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Clostridium difficile toxin A has been demonstrated to bind selectively to glycoconjugates with terminal Galα3Galβ4GlcNAc sequences (1). This binding property is shared with the monoclonal antibody Gal-13 (2) as well as 1% of circulating IgG in normal human blood (3). However, no Galα3-terminated glycoconjugates have been found in normal human tissue (4), except as part of the blood group B determinant.

A comparison of the binding of toxin A and the monoclonal antibody Gal-13 was undertaken, using mixtures of glycosphingolipids separated on thin-layer chromatograms (5). Both ligands bound to Galα3Galβ4GlcNAc-terminated glycosphingolipids, e.g. non-acid glycolipids from rabbit and bovine erythrocytes, as expected. In addition, a binding of toxin A to a glycosphingolipid in the pentaglycosylceramide region of the non-acid fraction from human erythrocytes was detected. This binding-active component was isolated and characterized by mass spectrometry, proton NMR spectroscopy and gas chromatography-mass spectrometry after degradation. Binding to serial dilutions of glycosphingolipids in microtiter wells (6) showed that the affinity of toxin A for the isolated pentaglycosylceramide was in the same range as for Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer, while the Gal-13 antibody only bound to the latter compound.

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S10.6

Glycobiology of Saliva — Clinical Relevance of an Oligosaccharide Mediated Host Defence System

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Salivary secretion is a complex fluid composed of a number of particular glycoconjugates possessing distinct structural and functional properties. In addition, they share general biochemical properties such as the occurrence of attached oligosaccharides. There is an increasing number of data supporting the existence of a specific host defence system at the mucosal surface mediated by oligosaccharides of salivary glycoconjugates irrespective of their peptide core. In this model the carbohydrate moiety of soluble mucus glycoconjugates inhibit competitively the lectin-mediated adhesion of microorganisms to the mucosa cell surface providing a first line of defence. According to this model stimulation of salivary secretion is a major challenge of this host defence system if the competitive oligosaccharides are diluted. To characterize the oligosaccharides in native saliva before and after stimulation of salivation, we have established a microtiterplate based test, which simulates the host defence model. Biotin-labeled lectins with known binding-specificity representing the microbial lectins bind to immobilized glycoproteins representing the mucosa cell glycoconjugates. Oligosaccharides attached to glycoconjugates of the sample can inhibit the carbohydrate mediated attachment of the labelled lectin to the immobilized oligosaccharides. For standardization the results are given in equivalents of a specific carbohydrate.

Using this test, we were able to show that in rats the oligosaccharide composition in saliva after pharmacological stimulation shows significant differences depending on the stimulating drug and that in a randomized subsample (N = 156) of Berlin citizens (Berlin Aging Study, BASE) stratified for age (70 – 105 years) and sex an age related increase in terminal mannose and *N*-acetyl-galactose occurs in unstimulated saliva. The comparison with clinical data in this group shows that gum recession is significantly lowered in persons who have a high activity of oligosaccharides in their saliva. Comparison of a group of patients with destructive periodontitis (N = 12) with a control group (N = 8) showed a significant reduction of terminal *N*-acetyl-galactose-residue in saliva only after stimulation measured by Vicia Villosa Agglutinin while in all other lectin tests no differences between the patient and a control group was detected.

S10.7

The Carbohydrate Chain Derived from Circulating Anodic Antigen Isolated from the Parasite *Schistosoma mansoni* is a Polysaccharide with Repeating -6)[Glc*p*Aβ(1-3)]Gal*p*NAcβ(1- UNITS

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Schistosomiasis is one of the most wide-spread parasitic infections of man, characterized by the persistent presence of adult *Schistosoma* worms in the portal and mesenteric veins. Antigen analysis plays an essential role to elucidate the immunological and immunopathological interactions between *Schistosoma mansoni* and its host. Despite the apparent importance of the glycoconjugate glycans in these antigenic reactions, little is known about their primary structures. Therefore, a study was initiated to elucidate the structure of the carbohydrate chains occurring in excretory gut-associated Circulating Anodic Antigen (CAA). CAA was isolated by immunoaffinity chromatography from adult *Schistosoma*