

mansoni worms. It was demonstrated that the presence of the exclusively *O*-linked carbohydrate chains are responsible for the antigenic character of CAA. Since no acidic non-carbohydrate substituents were detected, the negative charge has to arise from glucuronic acid. After reductive alkaline treatment, the *O*-linked carbohydrate chains were isolated by gel-permeation chromatography, and investigated by NMR spectroscopy. The analyses showed that the carbohydrate chains are unique polymers, consisting of $-6)[\text{Glc}\alpha\beta(1-3)]\text{Gal}\rho\text{NAc}\beta(1-$ repeating units.

S10.8

The Major *O*-Linked Carbohydrate Chain Derived from Circulating Cathodic Antigen from *Schistosoma mansoni* is a Poly- $\alpha(1-3)$ -Fucosylated Poly-*N*-Acetyllactosamine Polysaccharide

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Schistosomiasis is characterised by the persistence of adult *Schistosoma* worms in the portal and mesenteric veins of its host. The host humoral immune response includes an early and strong, reactivity against a number of gut-associated antigens. Such antigens are regularly released by the worm into the host's circulation when the parasite regurgitates the undigested contents of its gut. Immunodetection of the schistosome-specific gut-associated antigen Circulating Cathodic Antigen (CCA) is increasingly used for the quantitative diagnosis of active schistosomiasis.

In order to examine the structural basis for the antigenicity of CCA, the antigen was isolated from adult *Schistosoma mansoni* worms using immunoaffinity. The antigenic character of CCA was shown to reside in the *O*-linked carbohydrate chains, which were isolated following reductive β -elimination and fractionated chromatographically. The carbohydrate-containing fractions were analysed using 1-D and 2-D ¹H-NMR spectroscopy, fast atom bombardment mass spectrometry, and collision induced dissociation tandem mass spectrometry. These analyses revealed the presence of *O*-linked polysaccharides containing an average of eleven $-3)\text{Gal}\beta(1-4)[\text{Fuc}\alpha(1-3)]\text{GlcNAc}\beta(1-$ units. This fucosylated repeating unit is known as the Lewis X (Le^x) determinant. Reactivity of CCA with anti-CCA monoclonal antibodies was shown to depend on the fucosyl residues. In addition, a minor series of *O*-linked oligosaccharides was identified on CCA and the structures of these were also established.

S10.9

A 23 kDa Membrane Glycoprotein Bearing NeuNAc α 2-3Gal β 1-3 GalNAc *O*-Linked Carbohydrate Chains Acts as a Receptor for *Streptococcus sanguis* OMZ 9 on Human Buccal Epithelial Cells

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Streptococcus sanguis colonizes several human oral surfaces,

including both hard and soft tissues. Large salivary mucin-like glycoproteins bearing sialic acid residues have been reported to bind various *S. sanguis* strains. By contrast, the molecular basis for the adhesion of *S. sanguis* to human buccal epithelial cells (HBEC) has not been established. In a previous study, we have shown that *S. sanguis* OMZ 9 bound to exfoliated HBEC in a sialic acid-sensitive manner, the desialylation of these cells invariably leading to abolish *S. sanguis* OMZ 9 adhesion to their surface.

We here report that the resialylation of desialylated HBEC with the 2,3-sialyltransferase specific for *O*-glycans restored the receptor function for *S. sanguis* OMZ 9, whereas a similar cell resialylation with the 2,6-sialyltransferase specific for *N*-glycans had no effect. Moreover, the same resialylation reactions carried out by using CMP-9-fluoresceinyl-sialic acid yielded HBEC bearing fluorescence on the concerned acceptor membrane glycoproteins. A subsequent SDS/PAGE analysis revealed the occurrence of a single 23 kDa protein specifically labelled by fluorescent sialic acid residues, when resialylation was performed with the 2,3-sialyltransferase. This last finding demonstrates that this resialylated membrane glycoprotein which bears NeuNAc α 2-3 Gal β 1-3 GaNAc *O*-linked sugar chains and the receptor for *S. sanguis* OMZ 9 on HBEC are identical.

S10.10

Trypanosoma cruzi Acquires Exogenous Sialic Acid by Preferential Transfer to Flagellar Membrane Acceptor Sites

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Sialylation of galactose residues is mediated, in practically all eukaryotic cells, by sialyltransferases that use CMP-sialic acid as the monosaccharide donor. It was observed that “*in vitro*” (1,2) and “*in vivo*” (3), *T. cruzi* cells acquire sialic acid residues by trans-sialidase reaction using exogenous sialyl-containing molecules. Since there are evidences that the formation of sialyl-conjugates, on the external surface of the parasite, are required for invasion of mammalian cells “*in vitro*” (2), we decided to investigate the incorporation of sialic acid residues on the cell surface of *T. cruzi* grown in 10% rabbit serum. Different from other sera, such as mouse or fetal calf serum, the growth of the *T. cruzi* with rabbit serum leads to a strong agglutination reaction with Peanut Agglutinin and a markedly reduced agglutination with sialic acid-binding lectins, indicating that rabbit serum is a poor source of transferable sialic acid. Interestingly, microscopic examination revealed that *T. cruzi* agglutinates as small rosettes where contacts are exclusively located at flagella. Further incubation of these cells with 10% fetal calf serum induced a strong Wheat Germ Agglutinin reaction, involving contact cell bodies. These results suggest that under limiting substrate supply, sialic acid transfer occurs preferentially at the flagellum. We are currently investigating whether a similar distribution of sialic acid residues occurs in rabbit blood form trypomastigotes. These results may help elucidating basic aspects of *T. cruzi*-host cell interaction.

(1) Previato, J. O., Andrade, A. F. B., Pessolani, M. C. V. and Mendonça-Previato, L. (1985) *Mol. Bioche. Parasitol.*, **16**, 85–96. (2) Schenkman, S., Jiang, M. S., Hart, G. W.