L. Rydberg and P.-G. Nyholm
Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Hospital, Göteborg and Department of Medical Biochemistry, University of Göteborg, Göteborg, Sweden.

Human sera may in analogy to the blood group ABO system contain natural antibodies against carbohydrate structures that are lacking or hidden in single human individuals. Such antibodies are considered responsible for the hyperacute graft rejections seen in ABO incompatible organ transplantations. Accumulating evidence show that anti-carbohydrate antibodies are also responsible for the hyperacute rejections seen in xenotransplantation between discordant species as carbohydrate antigens expressed on different tissues seem to be the targets for such antibodies. As much as 1% of the total amount of human IgG antibodies are directed against a carbohydrate structure missing or hidden in humans i.e. Galα1-3Gal which is formally an equivalent to a defucosylated "linear" B-structure. This structure is present in many mammalian species like pig, rabbit etc. In persons of blood group B the Galα1-3Gal recognizing antibody does not interact with the B-trisaccharide. This led us to investigate whether there in persons of blood group A may exist antibodies recognizing the sequence GalNAcα1-3Gal ("linear" A) but not the A trisaccharide. We report the presence of natural IgM antibodies with this specificity. The antibodies can be detected after adsorption to and elution from a Synsorb^R column with GalNAcα1-3Gal carbohydrates attached and visualized with radioimmunoassay and chromatogram binding assay. The minimum energy conformations of the linear A and the linear B structures were calculated and compared to the conformations of the corresponding trisaccharides. The interactive properties of the saccharide chains were calculated with the GRID program. Attempts were made to define the binding surfaces of the linear oligosaccharide structures.

S12.10

Structural Analysis by GC/MS of Released Oligosaccharides from Glycosphingolipids from Different Organs of a Semi-Inbred Pig Strain

A. E. Bäcker[1] J. Holgersson[3], R. M. Binns[2] and B. E. Samuelsson[1]
[1]*Dept. Medical Biochemistry/Dept. Clin Chemistry and Transfusion Medicine, University of Göteborg, Sahlgrenska Hospital, Göteborg, Sweden*; [2]*AFRC Babraham, England*; [3]*Dept. Molecular Biology, Mass Gen Hosp, Boston, MA 02114, USA.*

Transplantation between species is seen as a possible solution to the lack of human donors. The pig is a possible source of donor organs in human xenotransplantation. A discordant xenotransplantation, e.g. pig to human, gives a hyperacute rejection of the organ within minutes, thought to be mediated by preformed IgM antibodies. Some of these IgM antibodies have been shown to react with the endothelium of the grafted organ and to have carbohydrate specificity (1). The expression of glycosphingolipids was characterized in different organs of a possible future pig donor strain, semi-inbred with respect to SLA and of blood group O.

The expression of non-acid and acid glycosphingolipids (gsphl) was studied in small intestine, liver, heart, salivary gland, kidney and spleen. The oligosaccharides were released by ceramide glycanase.The acid gsphl were purified into a total ganglioside and a total sulfatide fraction, and used for thin layer immuno-staining. The released non-acid oligosaccharides were analysed by gas chromatography (GC) and later by gas chromatography-mass spectrometry (GC/MS). Thin layer immuno-staining shows binding in some of the organs with anti-B (Dako) and TH-5, an anti-Galα1-3Galβ1-4GlcNAc-R antibody. The GC/MS verified the presence of a carbohydrate sequence in *e.g.* kidney consistent with the Gal 3nLc$_4$ structure. Human AB-serum was used to screen the fractions in order to look for the presence of IgM antibodies reacting with pig glycosphingolipids. A complex pattern was seen. Work in progress includes the characterization of protein-bound carbohydrates by Western Blot.

1 Platt, J. L. *et al.* (1990) *Transplantation.* **50**, 817 – 822.

S12.11

Involvement of Histo-Blood Group Antigens in the Susceptibility of Colon Carcinoma Cells to Natural Killer-Mediated Cytotoxicity

S. Ringeard[1], R. Zennadi[1], C. Goupille[1], M. Breimer[2], J. Harb[1] and *J. LePendu*[1]
[1]*CJF INSERM 90-11, Institute of Biology, Nantes, France*; [2]*Department of Surgery, University of Göteborg, Göteborg, Sweden.*

The susceptibility to NK cell lysis of human colon carcinoma cell lines was found to be inversely correlated to the expression of Lewis blood group antigens. Anti-Lewis monoclonal antibodies inhibited the NK or LAK-mediated cytotoxicity using Lewis positive cell lines as targets. In contrast, anti-MHC class I antibodies were not inhibitory at all despite high expression of the corresponding antigens on some cell lines. These results suggest that carbohydrate blood group antigens are either functional in the NK/LAK lysis phenomenon or closely associated to molecules involved when colorectal carcinoma cells are used as targets. Liposomes containing glycolipids bearing Lewis blood group structures or their precursors did not interfere with the NK/LAK-mediated cytolysis, suggesting that such carbohydrate structures present on the target cells are not recognized by lectins present on the effector lymphocytes. By immunoprecipitation and westem blotting experiments, we found that the Lewis antigens of various colon carcinoma cell lines were born almost exclusively by the $\alpha 6\beta 4$ integrin. It is thus likely that these carbohydrate structures interfere with the NK/LAK mediated cytolysis by modulating the function of the integrin.

S12.12

N-Glycanase, *O*-Glycanase and Endoglycosidase-F Treatment of Purified Tryptic Peptide of the Duffy Blood Group Antigen: Evidence for *N*-Glycosylation

K. Wasniowska, P. Eichenberger, F. Kugele and T. Hadley
Department of Medicine, University of Louisville and American Red Cross, Louisville, Kentucky.

Controversy exists in the literature concerning whether or not the protein carrying Duffy determinants (DP) on the red blood cell (RBC) is a glycoprotein. Tanner *et al.* found evidence for glycosylation; Chaudhuri *et al.* did not (1,2). We treated intact RBCs with *N*-glycanase (*N*-gly), Endoglycosidase-F (Endo-F) and *O*-glycanase (*O*-gly) and performed Western blots with an