S12.3

Round the World of Carbohydrate Antigens/Ligands in 80 × 10^{-4} Days!

T. Feizi
Glycoconjugates Section, MRC Clinical Research Centre, Harrow, UK.

Work with monoclonal antibodies has shown that there are spatio-temporal patternings in the display of several oligosaccharide antigens at the surface of cells and in extracellular matrices during stages of embryonic development and cell differentiation, such that there is a different repertoire of oligosaccharide antigens in each differentiated cell type. These repertoires change in a predictable way in malignancy. A major question has been to determine whether, as proposed (1), such oligosaccharides amount to 'area codes' that are decoded by proteins (endogenous lectins) which determine cell migration pathways, and whether they are ligands in macromolecular interactions and signalling in health and disease. Rapid progress in elucidating the roles of specific oligosaccharides (particularly those associated with glycoproteins) as recognition structures has been hampered in part by the lack of straightforward biological assay methods. To meet this challenge, we introduced a technology which enables oligosaccharide probes (neoglycolipids) to be generated from mixtures of oligosaccharides released from glycoproteins, and from desired structurally defined or chemically synthesized oligosaccharides. This has proven to be not only a powerful means of singling out, even from heterogeneous mixtures, the ligands for endogenous carbohydrate-binding proteins, but in addition it constitutes a microsequencing strategy for oligosaccharides, since the neoglycolipids have unique ionization properties in mass spectrometry. A striking development by this approach has been the discovery of a new class of oligosaccharide ligands that are *O*-linked and sulphate-containing for *E*-selectin (2) and are the most potent ligands so far for *L*-selectin (3), both adhesive proteins being directly involved in the recruitment and the pathways of migration of leucocytes in inflammation.

1. Feizi, T. *Trends Biochem. Sci.*, **6**:333 – 335 (1981); *Nature*, **314**:53 – 57 (1985).
2. Yuen, C. T., Lawson, A. M., Chai, W., Larkin, M., Stoll, M. S., Stuart, A. C., Sullivan F. X., Ahern T. J. and Feizi, T. *Biochemistry*, **31**:9126 – 9131 (1992).
3. Green, P. J., Tamatani, T., Watanabe, T., Miyasaka, M., Hasegawa, A., Kiso, M., Yuen, C.-T., Stoll, M. S. and Feizi, T. *Biochem. Biophys. Res. Commun.*, **188**:244 – 251 (1992).

S12.4

Glycopeptides as T Lymphocyte Antigens. Influence on Binding to MHC Class II and Immunogenicity

M. Meldal[1], I. Christiansen-Brams[1], K. Bock[1], S. Mouritsen[2] and O. Werdelin[2]
[1]*Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*; [2]*M&E, Dept. of Basic Immunology, 40 Lerso Parkallé, DK-2100 Copenhagen, Denmark.*

In order to investigate the immunogenicity of glycopeptides a peptide fragment from hen egg lysozyme, HEL(81-96)-Y (**1**), which is known to bind to the MHC class III molecule E^k but not A^k has been synthesized in 5 different glycosylated forms. The *N*-terminal serine of HEL(81-96)-Y was *O*-glycosylated with D-glucose (**2**), maltotriose (**3**) and a branched D-glucose pentasaccharide (**4**). Furthermore, HEL(81-96)-Y was prepared with a central serine or asparagine derivatized with the branched pentasaccharide (**5**) and *N*-acetyl-β-D-glucosamine (**6**) respectively. The ability of the five glycopeptides to bind the two MHC class II molecules was studied using a gel filtration assay. Neither **5** nor **6** were able to bind to any of the MHC class II molecules. Surprisingly **4** bound with larger affinity to MHC class II E^k than the parent peptide **1**. No binding to MHC class II A^k could be detected. The better binding to **4** was due to an increased association rate. The result of the binding study was supported by a functional study showing that **4** was able to elicit a heteroclitic proliferative response from T cells. Circular dichroism studies of **1** and **4** in mixtures of TFE and water indicated a less ordered structure of **4** and a predominant α-helical conformation of **1**. The CD-result was quantified by singular value decomposition. High affinity antibodies directed solely towards the pentasaccharide was raised with a synthetic glycoconjugate.

S12.5

Cloning and Sequencing of A cDNA Coding for Bovine Conglutinin

Y. Suzuki[1], Y. Yin[2], M. Makino[2], T. Kurimura[3] and N. Wakamiya[3]
Departments of [1]*Pathology, and* [2]*Microbiology, Osaka Prefectural Institute of Public Health, Higashinari, Osaka 537,* [3]*Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Suita Osaka 565, Japan.*

A 912 bp bovine cDNA fragment encoding bovine conglutinin was amplified by the RT-PCR technique. cDNA clones encoding the bovine conglutinin were isolated from a bovine liver cDNA library using a specific probe obtained from the PCR product. These cDNAs carry an insert of 1113 bp coding for a protein of 371 amino acid residues with a signal peptide of 20 residues. The deduced amino acid sequence of cDNA agrees with that determined by conventional amino acid sequence analysis. Two polyadenylation signal sequences were detected in the DNA sequence downstream of the 3′ end of the gene. Southern blot analysis of total bovine genomic DNA indicated that there is only one copy of the gene encoding bovine conglutinin. Northern blot analysis of bovine tissues showed that conglutinin mRNA of about 1.5 kb is expressed in the liver and also slightly in the lung.

S12.6

Xenotransplantation of Pig Islet Cells to Man: Specificities of Human IgM and IgG Anticarbohydrate Antibodies

L. Rydberg, T. Cairns, E. Karlsson, E. Möller, M. Satake, C.-G. Groth and B. E Samuelsson
Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska hospital, Göteborg; Department of Medical Biochemistry, University of Göteborg, Department of Clinical Immunology and Department of Transplant Surgery, Huddinge hospital, Stockholm, Sweden.

All humans have natural antibodies against pig tissues. Natural antibodies have been found often to be directed against carbohydrate structures. Such antibodies are of