S.8 CARBOHYDRATE-MEDIATED RECOGNITION

S8.1
Selectins, Sialosides, and Sialyltransferases

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Sialic acid containing carbohydrate groups of glycoproteins and glycolipids play diverse roles in biological recognition, serving as receptors for viruses, bacteria and microplasma as well as for cell adhesion molecules involved in cell recognition and cell trafficking. These recognition events are mediated by a diverse group of sialoside structures which are expressed in a cell type specific and developmentally regulated manner. The cellular control for selective expression of particular carbohydrate groups is only beginning to be explored, but appears to involve the regulated expression of the many glycosyltransferases involved in their synthesis. For example, six members of the sialyltransferase gene family which share a common 'sialyl motif' have been cloned[1-3], and each differs in its tissue specific expression, which appears to be regulated at the level of transcription[4,5]. Thus, regulation of sialyltransferase and other glycosyltransferase genes is likely to play a major role in the specificity involving intracellular recognition by carbohydrate binding proteins.

The role of the selectin family of cell adhesion molecules in leukocyte trafficking is undoubtedly one of the best documented examples of intracellular recognition and adhesion involving sialic acid containing carbohydrate groups. In particular, the E-selectin and P-selectin expressed on vascular endothelial cells and endothelial cells and platelets, respectively, participate in the recruitment of neutrophils to sites of inflammation and tissue injury via recognition of the carbohydrate group, sialyl Lewis X on the glycoprotein ligands of the neutrophil[6]. Although recruitment of neutrophils to sites of inflammation and tissue injury is a normal part of the immune response, occasionally the same mechanism results in excess recruitment of these cells resulting in tissue injury typified by diseases of acute inflammation and reperfusion injury (heart attack, stroke, and traumatic shock).

One approach to preventing the pathogenic consequences of these abnormal conditions is to block the adhesion molecules involved in neutrophil recruitment and migration into tissues. Several published studies suggest that blockade of E-selectin can be of therapeutic benefit[7,8]. Recent progress in the combined chemical and enzymatic synthesis of sialyl Lewis X based carbohydrate groups[9,10] have made possible testing of carbohydrate blockers of the selectins as inhibitors of inflammatory disease. Such observations raise the prospect for the practical application of complex carbohydrates in the treatment of human disease.

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S8.2
Carbohydrate-Carbohydrate Interactions of a Novel Class of Acidic Glycans Mediate Cell Adhesion and Recognition

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A new class of large fucosylated acidic glycans (AGs), distinct from the classical glycosaminoglycans, was isolated from embryonal and adult sponge, sea urchin and mouse tissues as well as from a variety of normal and malignant human cells. Anti-sponge AG monoclonal antibodies Block 1 and 2 were found to inhibit sponge cell adhesion as well as adhesion of sea urchin and human tumor cells expressing AGs. Beads coated with the purified protein-free sponge or sea urchin AGs showed a Ca^{2+}-dependent aggregation. Reconstitution of the sponge AG-AG binding was achieved by cross-linking AGs into polyvalent polymers of similar valency as in the native proteoglycans. These experiments suggested that the two interspecies carbohydrate adhesion epitopes intrinsic to AGs could mediate cell adhesion via the novel principle of polyvalent low affinity Ca^{2+}-dependent carbohydrate-carbohydrate interactions. This is in contrast to the cell adhesion molecules of cadherin, immunoglobulin, integrin and lectin gene families which operate through mono-tetravalent moderate affinity protein-protein or protein-carbohydrate binding. The structure of the sponge adhesion epitope recognized by the Block 1 antibody was recently shown by ^{1}H-NMR spectroscopy, fast atom bombardment-mass spectrometry, methylation analysis and sequential chemical and enzymatic degradation to be Pyr-4,6Galβ1-4GlcNAcβ1-3Fuc (Spillmann, D., Hard, K., Thomas-Oates, J., Vliegenthart, J. F., Misevic, G. N., Burger, M. M. and Finne, J. *J. Biol. Chem.* in press). Expression of adhesion glycan epitopes recognized by Block 1 and 2 could be related to major morphogenetic cell movements in sea urchins and to metastatic invasiveness of colon carcinomas in the human. *In vitro* studies on cell motility also demonstrated that sponge, sea urchin embryonal and human colon carcinoma cells increase migration up to 10 times on sponge AG substratums. These experiments suggest that differential spatial and temporal expression of various forms of adhesion AGs regulate initial cell adhesion, recognition and migration during embryogenesis and metastatic invasion.

S8.3
Site Directed Mutagenesis of the Carbohydrate Binding Region of *Erythrina corallodendron* Lectin