**C-Type Lectins and Sialyl Lewis X Oligosaccharides: Versatile Roles in Cell-Cell Interaction**

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**Cell Type–specific Carbohydrates**

Carbohydrates are major components of the outer surface of mammalian cells and these carbohydrates are very often characteristic of cell-types and developmental stages (Feizi, 1985; Hakomori, 1985). One of such cell type–specific carbohydrates is sialyl Lewis X, NeuNAcα2→3Galβ1→4(Fucα1→3)GlcNAc c–R. Sialyl Lewis X and Lewis X, Gα1→4(Fucα1→3)GlcNAc c, were originally discovered as differentiation antigens specific to granulocytes and monococytes (Fukuda et al., 1984; Mizoguchi et al., 1984). These oligosaccharides can be synthesized when α1,3-fucosyltransferase(s) is present (Maly et al., 1996). Erythroid cells, which lack the α1,3-fucosyltransferase, in contrast, express α1,2-fucosyltransferase. Resultant H-type oligosaccharides (O blood type), Fucα1→2Galβ1→4GlcNAc cβ1–R, are converted to A and B blood group antigens by addition of α,1,3-linked N-acetylgalactosamine or galactose through erythroid cell specific expression of two unique glycosyltransferases (Hakomori, 1985). These results show a typical example of cell type–specific oligosaccharides, of which synthesis is dependent on cell type–specific expression of a unique glycosyltransferase.

Specific expression of unique oligosaccharides strongly suggests that these oligosaccharides may serve as cell surface markers. Indeed, rapid and explosive understanding of the roles of sialyl Lewis X and its variants as cell recognition molecules has been taking place recently. In this mini-review, we would like to focus on roles of this group of carbohydrates and C-type lectins that recognize those carbohydrates (for other aspects on this subject, see Springer, 1994; Butcher and Picker, 1996).

In mammals, lymphocytes circulate in the vascular and lymphatic compartments, allowing maximum exposure of lymphocytes to foreign pathogens. Lymphocytes leave the vascular compartment at lymph nodes, traverse the lymphatic organs, and then return to the vascular system. This directed flow of lymphocytes is dependent on carbohydrate ligands present on specialized endothelial cells, termed high endothelial venules (HEV)1. It was discovered that lymphocyte binding to HEV is dependent on sialic acid on HEV and can be inhibited by fucosylated sulfated oligosaccharides (Rosen and Bertozzi, 1996). When the homing receptor on lymphocytes, now called L-selectin, was molecularly cloned, its cDNA sequence predicted a carbohydrate-binding domain at its NH2-terminus. This carbohydrate-binding domain is similar to that of the hepatic lectin, which recognizes asialo plasma glycoproteins (A shwell and H arford, 1982). Carbohydrate-binding activity of these lectins is dependent on Ca2+, thus they are collectively called C-type lectin (Drickamer, 1994). Counter-receptors on HEV capture circulating lymphocytes via L-selectin–dependent adhesion, leading to transmigration (Fig. 1). L-selectin was found to be required for this process (A rbones et al., 1994).

In parallel to this, two other adhesion molecules playing critical roles in the interaction between leukocyte-endothelial cells were identified. One of them, P-selectin, appears on the cell surface of platelets upon stimulation. Similarly, P-selectin, then E-selectin, another adhesive molecule, appears on the cell surface of endothelial cells after stimulation by inflammatory agents. E- and P-selectin also contain a carbohydrate-binding domain, which is highly homologous to that of L-selectin, having ~60% identity at the amino acid levels among these molecules (Springer, 1994).

Once these adhesion molecules were recognized as carbohydrate-binding proteins, carbohydrate-ligands for E- and P-selectin were immediately identified as sialyl Lewis X (Lowe et al., 1990, for others, see Springer, 1994), previously shown to be present on neutrophils. During inflammation, leukocytes expressing sialyl Lewis X are recognized by P- or E-selectin and such initial adhesion results in the slowing down of leukocytes, a “rolling effect.” This rolling effect leads to vascular extravasation of leukocytes, similar to the process shown in Fig. 1.

**Specificity in Selectin-Carbohydrate Interaction**

The above results so far indicate that all selectins recognize sialyl Lewis X as ligands. How then could each interaction achieve a more specific interaction?

First, glycoproteins presenting sialyl Lewis X oligosaccharides are specific for each cell-type, providing unique interactions to each cell-type. L-selectin carbohydrate ligands are presented by GlyCAM-1, CD34, MADCAM-1, and other glycoproteins, all of which contain mucin-type O-linked oligosaccharides. P-selectin carbohydrate ligands
endothelial cells at HEV. Activation of integrins may be separately achieved through the change in cytoplasmic tail of L-selectin upon its binding to the ligands. A portion of L-selectin molecules is shed after proteolysis (Kahn et al., 1989).
may be present in N-glycans or glycans resistant to digestion with O-sialylglycoproteinase, which cleaves mucin-type glycoproteins (Clark et al., 1998). A addressing these issues will likely reveal entirely new aspects of selectin ligands.

The second point is that sulfation of sialyl Lewis X in HEV is more intrinsically regulated than we know now. Structural analysis of GlyCAM-1 oligosaccharides indicated the presence of 6'-sulfo sialyl Lewis X, NeuNAα2→3Galβ1→4GlcNAc→R and possibly 6,6'-bisulfo sialyl Lewis X, NeuNAα2→3(sulfo)Galβ1→4(sulfo)GlcNAc→R, in addition to 6-sulfo sialyl Lewis X, in core 2 branched oligosaccharides (Fig. 2, see Hemmerich et al., 1995). 6'-Sulfo sialyl Lewis X expressed on the cell surface supported the adhesion to L-selectin (Tsuboi et al., 1996). A disulfated form of lactose inhibited L-selectin binding to GlyCAM-1 better than its monosulfated form (Bertozzi et al., 1995). MECA-79 antibody that specifically detects HEV and inhibits the L-selectin-mediated binding in vivo and in vitro (Streeter et al., 1988), recognizes a sulfated form of mucin-type O-glycans (Hemmerich et al., 1994). Expression of LSST or GlcNAc 6-sulfotransferase in combination with other known sulfotransferases failed to form the MECA-79 antigen (Hiraoka et al., 1999). These results suggest that 6,6'-bisulfo sialyl Lewis X or other mono- and multiple sulfated sialyl Lewis X may play a role as an L-selectin ligand. It will be important to identify additional sulfotransferases that form such L-selectin ligands.

**Versatile Roles of C-type Lectin in Cell–Cell Interactions**

The roles of C-type lectins is not limited to selectin-carbohydrate interactions. Indeed, various receptors were demonstrated as C-type lectins such as an IgE Fc receptor (FcyRII) and pulmonary surfactant (Drickamer, 1994). A C-type lectin domain of NK cell receptor(s) was shown to bind to fucoidan (Matsumoto et al., 1998), which is also an inhibitor for L-selectin binding. It was also shown that melanoma cells densely expressing sialyl Lewis X oligosaccharides in short N-glycans after the transfection with an α1,3-fucosyltransferase can be targeted by NK cells, most likely through NK cell receptors of C-type lectin (Ohyama et al., 1999). On the other hand, B16 melanoma cells, which expressed moderate amounts of sialyl Lewis X in poly-N-acetyllactosamines long chain glycans after the same transfection, were highly metastatic probably through interaction with a C-type lectin on lung endothelial cells (Ohyama et al., 1999). This finding is consistent with the previous reports that carcinoma cells are enriched with sialyl Lewis X in poly-N-acetyllactosamines (Hakomori, 1985). These results provide a clear-cut example that a subtle difference in carbohydrate ligands results in entirely different biological consequences.

The carbohydrate-recognition domain of a NK cell receptor binds to either MHC class I peptide or fucoidan (Matsumoto et al., 1998). Similar and dual binding of C-type lectins was demonstrated in C-type lectin domains of proteoglycans such as brevican. In one of these cases, the C-type lectin domain binds to tenasin-R (Aspberg et al., 1997) or HNK-1 glycan (Miura et al., 1999), another sulfated glycan uniquely present in neural and NK cells. The binding to one may preclude another from binding, providing another example of dual recognition by a C-type lectin. We expect that more examples will follow.

In summary, this overview presents clear examples where carbohydrate-protein (C-type lectins) interaction plays a critical role in cell–cell interaction. The results demonstrate that the interaction of sialyl Lewis X oligosaccharides with a specific C-type lectin plays a critical role in cell–cell interaction. At the same time, modification, such as sulfation of sialyl Lewis X, its multiple presentation, scaffold of carbohydrates and the structure of the glycoprotein itself, all contribute to the specificity of the interaction, which ultimately regulates the biological function of sialyl Lewis X. Efforts to identify the precise structure of oligosaccharides recognized by a given C-type lectin involved in each case will provide critical understanding for the roles of oligosaccharides in cell–cell interactions. Such studies undoubtedly will enhance our understanding of the mechanisms dictating how cell–cell interactions can be finely tuned.

The authors thank Drs. John B. Lowe, Jamey D. Marth, Ole Hindsgaul, Michiko N. Fukuda, and Jun Nakayama for continuous productive collab-
ative efforts with us, Dr. Edgar Ong for critical reading of the manuscript, and Susan Fanno for organizing the manuscript.

Submitted: 14 September 1999
Revised: 24 September 1999
Accepted: 29 September 1999

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