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The Selectins - A family of sialic acid-binding lectins directly involved in intercellular adhesion

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It has been an article of faith among students of glycoconjugates that specific carbohydrate sequences must be involved in specific types of intercellular adhesion. However, to date there have been very few bonafide examples of such specificity. The recent profusion of articles on the "selectin" family of carbohydrate-binding proteins should therefore be of interest to readers of this newsletter. The pace of publication in this "hot" area has been fast and furious. Consequently, while many excellent studies have been published, erroneous or misleading information has also been put out. Recent reviews on the subject (Brandley, B. K. et.al. *Cell* **63**, 861-863,1990; Springer and Laskey *Nature* **349**, 196-197, 1991) were obliged to quote many articles still "in press", and to draw several speculative conclusions. The present commentary suffers from the same problems, and may well be out of date by the time it is published!

The "Selectins" or "LEC-CAMs" are a closely-related family of cell surface molecules that recognize and bind to other cell types in a calcium-dependent manner. They share homologous N-terminal sequences that have "carbohydrate recognition domains" similar to those originally identified by Drickamer in other "C-type" mammalian lectins. The recent and extensive literature on the biology,cloning, expression and molecular structure of these proteins will not be reviewed here. This brief commentary will deal strictly with recent evidence regarding the structure of the carbohydrate ligands

recognized by these proteins. Because of the numbers of papers involved, it is not possible to evaluate each study in detail here. Rather, an overview of the experimental approaches taken is provided, along with a summary of the current status of knowledge. The following types of experimental evidence have been used to prove the nature of the carbohydrate ligands for these receptors:

Conclusive Evidence

(a) **Direct binding of soluble forms of the selectins to defined carbohydrate structures from the cell bearing the ligand.** This is the strongest type of evidence for specificity. Once such information is available, related model compounds can be used to further define the specificity of the interaction.

Strong Evidence

(b) **New expression of the carbohydrate ligand on host cells upon transfection with a specific glycosyltransferase cDNAs**. If the precursor structures existing in the host cells are relatively well-known, the product of the newly expressed transferase can be predicted. Assays of cell extracts for the enzyme can confirm that expression was successful.

(c) **"Expression cloning" of the unknown ligand results in isolation of a specific glycosyltransferase cDNA.** The derived amino acid sequence of a cDNA isolated in this manner was found to have high homology to a previously sequenced glycosyltransferase. Again, assay of cell extracts for the newly synthesized enzyme confirmed that expression was successful.

(d) **Destruction of the carbohydrate ligand using specific glycosidases**. This is strong presumptive evidence for the nature of the structure involved in the interaction. However, lack of destruction of the ligand by a glycosidase does not necessarily rule out specific structures, since resistance could result from a variety of reasons. Furthermore, glycosidase preparations can have other activities that could confuse the result (e.g. Newcastle disease virus has a cell-agglutinating activity as well as an α2-3 specific sialidase activity, and some commercial enzymes can be contaminated with proteases). Only in some reports were the appropriate controls done using specific inhibitors of the glycosidases.

(e) **Synthesis of the ligand <u>in vitro</u> using specific glycosyltransfereases.** When the precursors and products are well understood, this approach is quite reliable. It has also permitted generation of closely related structural variants that can be evaluated as potential ligands for the receptor.

(f) **Direct binding of intact cells expressing the receptor to immobilized glycoconjugates with known structures**. Transient expression of the receptor in COS cells gives very high levels of surface

receptor at a reasonable efficiency. This has permitted such experiments to be carried out.

(g) **Binding of mutant lectin-resistant cells expressing novel carbohydrate structures to purified, immobilized receptors.** Similar studies can be done by direct binding of the mutant cells to intact endothelial cells bearing the receptors. The wild-type precursors of the lectin-resistant cells serve as controls.

Weak evidence

(h) **Inhibition of binding by specific carbohydrate structures.** In general, soluble monovalent carbohydrates are poor inhibitors of multivalent reactions involving lectins and cell surfaces. Thus, such results only provide general clues regarding the interaction, and must not be overinterpreted. Multivalent carbohydrates may give better inhibition, but tend to be heterogenous with regard to their structure.

(i) **Inhibition of binding by specific monoclonal antibodies or lectins.** While this approach sounds very specific, it suffers from the fact that antibodies and lectins are relatively large molecules, that can coat cell-surfaces and interfere non-specifically with cell-cell interactions (this is especially true of IgM immunoglobulins, which account for most of the available anti-carbohydrate antibodies). Furthermore, antibodies and lectins are themselves multivalent and can agglutinate cells, making evaluation of cell-cell interactions difficult.

The table summarizes the current status of published knowledge regarding the carbohydrate ligands for these receptors, and is based upon the 10 recent papers cited above. Many lines of evidence show that the ligand for ELAM-1 is sialyl-Lewis X or Siaα2-3Galß1-4(Fucα1-3)GlcNAcß1-R. This structure appears to be both necessary and sufficient for binding by this receptor, regardless of whether it is carried on glycolipids, O-linked oligosaccharides or N-linked oligosaccharides. This terminal sequence has been found on cells of the myeloid lineage, on various types of cancer cells, and on certain types of mucins. The structure of the ligands for the other two receptors are far less clear at the present. However, they both clearly involve sialic acids. Thus at the present time, it can be concluded that these these proteins are a family of sialic acid-binding lectins, whose specificity is defined further by other aspects of the oligosaccharide structure (e.g. the Lewis X motif in the case of ELAM-1). Since many different types of sialic acids and terminal oligosaccharide sequences are known, there are many candidate structures to choose from. It must be kept in mind that more than one related structure may prove to be a ligand for each of these receptors, and that their relative affinities might vary. It also remains possible that the carrier of the oligosaccharide (protein or lipid) could contribute to the specificity in some cases.

This information is of great importance in understanding normal and abnormal cell-cell interactions in a variety of situations, and has great potential for therapeutic manipulations. Thus, one can predict that the current pace of activity in this area will continue. If so, many of the remaining issues regarding the carbohydrate ligands of the selectins may well be resolved by the end of 1991.

RECEPTOR (synonyms)	CELLS CARRYING THE		TYPES OF EVIDENCE*	STRUCTURAL FEATURES OF THE LIGAND	
	RECEPTOR	LIGAND		NECESSARY FOR BINDING	SUFFICIENT FOR BINDING
MEL-14 antigen (gp90MEL, LAM-1, Leu8,Ly22)	Lymphocytes, Neutrophils	Endothelial cells	d,g,h,i	Sialic Acid(s) ?Sulfate esters ??Phosphate esters	Sialyl-?
ELAM-1	Endothelial cells	Neutrophils, and some lymphocytes?	a,b,c,d,e, f,g,h,i	Sialic Acid Fucosylated polylactosmine chains (on glycolipids or glycoproteins)	Sialyl-Lewis X?
GMP-140 (PADGEM, CD62)	Platelets, Endothelial cells	Neutrophils, Monocytes	d,h,i	Sialic Acid(s) ?on glycoproteins only ?endo-ß-galactosidase resistant structure ??Lewis X structure	Sialyl-?