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CELL ADHESION

Sticky sugars for selectins

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CELL-adhesion research has suddenly become sweeter. Only a year after three members of a family of cell-adhesion molecules were found to have lectin-like N-terminal domains[1,2], a spate of reports has appeared identifying the sticky sugar structures that are their putative ligands (see figure). That a good portion of these papers come from biotechnology companies reflects the fact that carbohydrate cell-adhesion ligands are relatively small, simple structures; thus the synthesis of drug analogues that will be tested for anti-inflammatory and anti-thrombogenic properties cannot be far off.

The role of carbohydrates in cell interactions started to receive close attention with the recognition that proteoglycans and glycosaminoglycans are important components of the extracellular matrix. Heparan, chondroitin sulphate and hyaluronic acid bind to many proteins in the matrix. In theory, carbohydrates are well suited for specific recognition — monosaccharides have many hydroxyls that can be linked *O*-glycosidically in both branched and linear arrays, so far more carbohydrate than protein structures can be created from the same number of monomers. Protein–protein recognition has received most of the limelight in studies of cell–cell adhesion, because this is the dominant and perhaps exclusive form of recognition by adhesion receptors of the integrin and immunoglobulin superfamilies[1]. But work on carbohydrates has advanced rapidly with the determination of many complex structures by fast-atom-bombardment mass spectrometry, and of cell type-specific carbohydrate structures and linkages that are regulated during cell differentiation by selective expression of glycosyl transferases. Recent examples of the identification of carbohydrate receptors on cell surfaces include one on sperm which recognizes an *O*-linked carbohydrate in the zona pellucida[3]; and CD44, a widely distributed surface receptor for hyaluronic acid[4,5].

Now a family of adhesion-receptor glycoproteins critical to interactions of circulating cells within the vasculature must be added to the list. This family has been designated the selectins (ref. 6) or LEC-CAMS (ref. 2). The name selectin capitalizes on the derivation of 'lectin' and 'select' from the same Latin root, meaning to separate by picking out, and is in keeping with those of the immunoglobulin, integrin and cadherin adhesion-receptor families. The homing receptor selectin, also called gp90^{mel}, LAM-1, LEC-CAM-1 or LECAM-1, is expressed on leukocytes and facilitates their binding to endothelium during lymphocyte recirculation through peripheral lymph nodes and neutrophil emigration at inflammatory sites. The PADGEM, GMP-140 or CD62 molecule is a granule-associated glycoprotein of platelets and endothelial cells that is brought to the cell surface after stimulation by thrombogenic agents, allowing these cells to bind neutrophils and monocytes at the site of tissue injury. The ELAM-1 glycoprotein is synthesized by endothelial cells in response to inflammatory agents and promotes adhesion of neutrophils, monocytes and a subpopulation of lymphocytes. These proteins thus mediate adhesion of leukocytes to endothelium and platelets during inflammation and clotting[1,2,7].

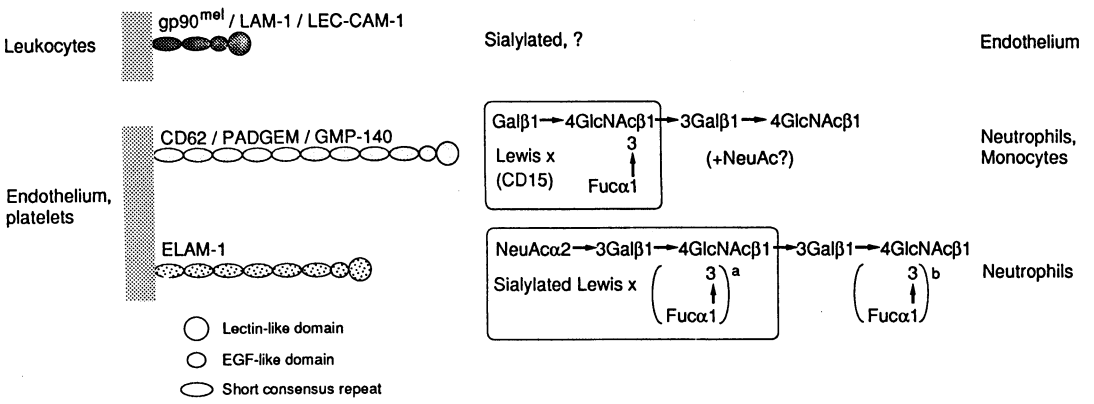
Clues that the selectins are carbohydrate-specific came from two directions. Early studies had shown that the homing receptor selectin could bind to algal or yeast polysaccharides rich in fucose sulphate or mannose phosphate; that these and related monosaccharides could competitively inhibit binding to lymph-node endothelial cells; that binding is Ca^{2+}-dependent; and that binding is abrogated by neuraminidase treatment of the endothelial cells[7]. Subsequent cloning of complementary DNAs revealed that the homing receptor, ELAM-1, and CD62 are members of a structurally related adhesion-receptor family that has an N-terminal domain homologous to Ca^{2+}-dependent (C-type) animal lectins (see figure). Some animal lectins are found at cell surfaces, and include hepatic receptors that are specific for terminal galactose and fucose[8]. Although the homing receptor was the first selectin thought to have a carbohydrate ligand[7], the identity of the biological ligand remains unclear; the recent results concern the ligands of the other two selectins, CD62 and ELAM-1.

The CD62 ligand, known to be present on neutrophils and monocytes, was putatively identified by screening for monoclonal antibodies that would block binding of these cells to CD62. CD15, also known as Lewis x (see figure), was thus identified as a component of the ligand, and this was supported by the ability of a purified Lewis x adduct of lactose found in human milk to partially inhibit adhesion at relatively high concentrations[9].

No less than five independent papers have appeared within the past three months on the structure of the ELAM-1 ligand and the enzymes that synthesize it. Neutrophils are rich in Lewis x and sialylated Lewis x (refs 10 and 11; see figure). These are $\alpha(1\rightarrow3)$ fucosylated derivatives of polylactosamine that are found at the nonreducing termini of glycolipids, *O*-linked carbohydrates and *N*-linked oligosaccharides. Expression of sialylated Lewis x in neutrophils and a variety of cell lines, including mutants with altered $\alpha(1\rightarrow3)$ fucosylation, correlates with ELAM-1-dependent adhesiveness[12–14]. Furthermore, monoclonal antibodies specific for sialyl Lewis x, as well as a difucosylated sialyl Lewis x glycolipid or sialyl Lewis x-containing mucin added as competitors, inhibit binding of neutrophils to endothelial cells or a purified ELAM-1 chimaeric globulin[13,14]. Negative cells can be converted to ELAM-adhesive cells positive for sialyl Lewis x by transfection with an $\alpha(1\rightarrow3/1\rightarrow4)$-fucosyl transferase that is the product of the Lewis blood group locus[12]. A distinct fucosyl transferase, with a more restricted $\alpha(1\rightarrow3)$ specificity and an expression limited to myeloid cells that bind ELAM-1, was isolated by expression cloning, using a monoclonal antibody to an undefined carbohydrate structure that acts as an ELAM-1 ligand[15]. Transfection showed that adhesion of cells to ELAM-1 can be regulated by expression of this single sugar transferase.

In a daringly direct approach to ligand identification, a different group fractionated a glycolipid extract of neutrophils and assayed fractions for binding to ELAM-transfected cells[16]. Mass spectrometry of a highly purified fraction demonstrated a structure similar to sialyl Lewis x, except that fucose is in $\alpha(1\rightarrow3)$ linkage with the penultimate rather than the ultimate *N*-acetyl glucosamine (see figure). The sialylated, penultimately fucosylated structure is identical to CD65 (ref. 17), expression of which was shown by another group not to correlate with ELAM-1 binding[14]. But the difucosylated structure clearly binds[13], and all groups agree that enzymatic removal of sialic acid or fucose ablates ligand activity. In summary, ELAM-1 recognizes a family of sialylated, fucosylated polylactosamines, and the critical determinant seems to be sialyl Lewis x.

Although these results forcefully and elegantly demonstrate the role of carbohydrates as specific mediators of cell adhesion, many issues remain to be resolved. The structures identified so far may represent only the minimum requirements for selectin binding; given the enormous diversity inherent in carbohydrates, alternative minimal structures, or more complex structures containing substituents that increase affinity, might be found. A carbohydrate structure containing sialic acid seems to have higher affinity than the unsialylated Lewis x structure for CD62, because neuraminidase treatment of neutrophils reduces CD62-mediated adhesion[18,19]. Uncertainty about the exact



Selectins and their ligands. The selectins have N-terminal lectin-like domains, an epidermal-growth-factor-like domain, and varying numbers of short consensus repeats as found in proteins regulating complement activation[1,2]. Fucoses in parentheses in the ELAM-1 ligand: fucosylation was reported at sites marked a (ref. 14), a or a and b (ref. 13), and b (ref. 10). NeuAc, sialic acid; Gal, galactose; GlcNAc, *N*-acetyl glucosamine; Fuc, fucose.

relationship between Lewis x (CD15) and the CD62 ligand arises from the incomplete inhibition by antibodies to CD15 of binding to purified CD62 in the original report[9], and a failure of a different monoclonal antibody to CD15 to inhibit this interaction in a subsequent report[19].

That there coexist on the cell surface subpopulations of ligand structures that differ in affinity, or in accessibility owing to the nature of their linkage to the cell surface, is suggested by the observation that neutrophil activation decreases adhesiveness for ELAM-1 (ref. 20) without decreasing binding sites for sialyl Lewis x monoclonal antibody[14]. Ligand structures also seem to differ from cell to cell. The ELAM-1 ligand structures on natural killer cells and granulocytes are not the same, because although both cells express sialylated Lewis x (ref. 21) and bind to ELAM-1 (ref.15), a monoclonal antibody that binds to a carbohydrate determinant on the ELAM-1 ligand on granulocytes does not bind to natural killer cells[15]. This antibody has a specificity distinct from monoclonal antibodies specific for sialylated Lewis x, and probably binds to a less terminal carbohydrate, because its determinant does not involve sialic acid. Still another ligand seems to exist on a subpopulation of skin-homing memory lymphocytes — these cells bind to ELAM-1-transfected cells[22,23], yet lymphocytes do not express sialylated Lewis x (ref. 21). The determinants of receptor specificity and affinity may be overlapping but distinct from determinants of antibody specificity, and both are likely to be structurally heterogeneous.

At their meetings, glycobiologists often give one another pep talks about the biological role of glycoconjugates. The many oligosaccharide structures attached to proteins or lipids that have been determined over the past decades, sometimes without a clue as to their functions, have led to a crowning achievement in the field of cell adhesion. Interesting refinements or variations in the elegant yet simple structures of the two reported selectin ligands, and identification of the third, will probably obsess researchers in this suddenly hectic field before they can turn to the design of synthetic analogues that could result in pills that are sugar-loaded as well as sugar-coated. Adhesion inhibitors are promising candidates for anti-inflammatory and anti-thrombogenic drugs. And because sialyl Lewis x is expressed on diverse tumour types[24], including colon carcinoma cells that bind to ELAM-1 (ref. 25), such drugs might also be anti-metastatic. □

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1. Springer, T. A. *Nature* **346**, 425–433 (1990).
2. Stoolman, L. M. *Cell* **56**, 907–910 (1989).
3. Wassarman, P. M. *Development* **108**, 1–17 (1990).
4. Miyake, K., Underhill, C. B., Lesley, J. & Kincade, P. W. *J. exp. Med.* **172**, 69–75 (1990).
5. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C. B. & Seed, B. *Cell* **61**, 1303–1313 (1990).
6. Bevilacqua, M. P. *Circulation* **80**, ii–i (1989).
7. Yednock, T. A. & Rosen, S. D. *Adv. Immun.* **44**, 313–378 (1989).
8. Drickamer, K. *Biochem. Soc. Trans.* **17**, 13–15 (1989).
9. Larsen, E. *et al. Cell* **63**, 467–474 (1990).
10. Fukuda, M., Spooncer, E. S., Oates, J. E., Dell, A. & Klock, J. C. *J. biol. Chem.* **259**, 10925–10935 (1984).
11. Symington, F. W., Hedges, D. L. & Hakomori, S.-I. *J. Immun.* **134**, 2498–2506 (1985).
12. Lowe, J. B. *et al. Cell* **63**, 475 — 484 (1990).
13. Phillips, M. L. *et al. Science* **250**, 1130–1132 (1990).
14. Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M. & Seed, B. *Science* **250**, 1132–1135 (1990).
15. Goelz, S. E. *et al. Cell* **63**, 1349–1356 (1990).
16. Tiemeyer, M. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Macher, B. A., Buehler, J., Scudder, P., Knapp, W. & Feizi, T. *J. biol. Chem.* **263**, 10186–10191 (1988).
18. Corral, L., Singer, M. S., Macher, B. A. & Rosen, S. D. *Biochem. biophys. Res. Commun.* **172**, 1349–1356 (1990).
19. Moore, K. L., Varki, A. & McEver, R. P. *J. Cell Biol.* (in the press).
20. Gimbrone, M. *et al. Science* **246**, 1601–1603 (1989).
21. Ohmori, K. *et al. Blood* **74**, 255–261 (1989).
22. Picker, L. J., Kishimoto, T. K., Smith, C. W., Warnock, R. A. & Butcher, E. C. *Nature* (in the press).
23. Shimizu, Y. *et al. Nature* (in the press).
24. Fukushima, K., Hiroto, M., Terasaki, P. I. & Wakisaka, A. *Cancer Res.* **44**, 5279–5285 (1984).
25. Rice, G. E. & Bevilacqua, M. P. *Science* **246**, 1303–1306 (1989).