Glycoconjugate Journal (1991) **8**:381–386

GLYCOPINION

Editor: RAYMOND A. DWEK

Many cell surfaces are covered by a glycocalyx composed of glycoproteins, glycolipids or glycosaminoglycans. These are frequently large, heavily glycosylated molecules ideally placed to mediate initial contacts between cells. The limited number of monosaccharide residues which constitute the oligosaccharide chains may be assembled into many different structures by means of different linkages, by chain branching and by the presence of substituents such as sulphate, phosphate or acetyl groups. These structures can be generated by relatively few enzymes allowing an impressive array of diversity to be regulated by a small number of gene products. The amount of information contained in such molecules suggests that they may have a role in events such as cell adhesion and cell trafficking.

The major issues raised in this month's provocative mini-review by Jeffrey Winkelhake of The Cytel Corporation are intended to create a forum for exploring questions of whether the newly-discovered complex carbohydrates involved in leukocyte trafficking and adhesion to vascular endothelium could provide valuable therapeutics for a wide range of inflammatory diseases and conditions.

The questions raised include:

- Are the complex carbohydrate ligands associated with leukocytes and endothelial cells in lymphoid organs restricted in their expression among sub-populations of cells?
- Or, are just a few oligosaccharide structures shared among leukocytes and endothelial cells? In this regard, how selective are the 'selectins'?
- Is there a reason why inflammatory signals can cause both up- and down-regulation of selectins? Will this and other phenomenon discovered *in vitro* hold up as research studies move *in vivo*?
- Is there meaning to the apparent time-dependent sequence of appearance of the carbohydrate-specific adhesion molecules, and does the sequence of expression predict an important role for each selectin in different inflammatory states?
- Since inflammatory cell trafficking is a multifaceted process involving adhesion, transcellular migration and several cell activation signals as well as several adhesion molecules simultaneously, will reagents designed to block selectin–ligand interactions provide meaningful therapeutics?
- Finally, since the carbohydrate ligands recognized by selectins are constitutively expressed, will therapeutics designed to block their recognition by selectins increase host susceptibility to infection or block other normal homeostatic mechanisms?

MINI-REVIEW

Will complex carbohydrate ligands of vascular selectins be the next generation of non-steroidal anti-inflammatory drugs?

JEFFREY L. WINKELHAKE

Cytel Corporation, 3525 John Hopkins Ct, San Diego, CA 92121, USA

Keywords: adhesion; carbohydrates; lectins; inflammation therapeutics; platelets; endothelial cells; leukocytes; homing or trafficking

Abbreviations: CAM, cell adhesion molecule; CD-, cluster of differentiation, number applied to leukocyte cell surface antigens; EGF, epidermal growth factor; IL-1, interleukin-1; LAD, leukocyte adhesion deficiency; LEC-CAM, member of family of CAMs characterized by having a lectin, EGF and complement-binding domains = selectin; Lex = LNF-III = lacto-n-fucopentaose III (see Fig. 1); NSAID, non-steroidal, anti-inflammatory drug; SLex, sialylated Lewis X antigen (see Fig. 1).

0282-0080/91 $03.00+.12

It has long been recognized that the adhesion of circulating leukocytes to vascular endothelial cells is a key step in inflammatory responses to infectious agents. Even though many of the pathologic alterations associated with inflammation are likely to be secondary to cell adhesion, the attachment of phagocytes to vessel walls via adhesion receptors or cell adhesion molecules (CAMs), is a necessary and early step in most acute inflammatory processes such as septic shock [1], appendicitis [2], acute alveolitis [3], the late-phase response in asthma [4, 5] and diseases associated with Arthus- and Schwartzman-type reactions [6, 7].

The appearance of CAMs in glomerulonephritis [8], arthritic joints [9, 10], psoriatic and other inflamed cutaneous tissues [11, 12], the blood–brain barrier in multiple sclerosis [13, 14], the intestinal mucosa in inflammatory bowel disease [15] and in atherosclerotic plaques [16] all suggest significant roles for endothelial CAMs in chronic-type inflammatory diseases as well. Furthermore, even though initially considered a non-thrombogenic barrier, the vascular endothelium and, specifically, CAMs have more recently been recognized as key players in haemostasis [17], reperfusion injury [18] and haemorrhagic shock [19]. Finally, since endothelial cell adhesion molecules are up-regulated as a result of surgery and organ transplantation [20] and often involved in tumor cell metastasis [21–24] and virus, bacteria and other pathogen attachment to cells [25–28], there is little doubt that CAMs will be the subject of therapeutic targeting in the years to come.

The newest CAMs to be discovered consist of a family of molecules each of which contain extracellular structures. These include a C-type (Ca^{2+}-dependent) lectin-like domain, an epidermal growth factor domain and several repeats of complement binding domains; thus, the descriptive acronym 'LEC-CAM'. While the major focus has been on the biological relevance of the LEC-CAM's lectin-like specificity, thus the evolution of the anthropomorphic acronym 'Selectin', it is undoubtedly important that this family of three 90–140 kDa cell surface glycoproteins have repeating structural motifs which resemble (have ca 80% homology with) both the C-regulatory proteins which bind C3 and C4, and the IL-2 receptor and serum factor XIII [29]. The LEC-CAMs are also extensively glycosylated, and variability in the extent and/or type of oligosaccharides attached could contribute to important selectin interactions with other receptors or even with each other.

The LEC-CAMs each bind complex, anionic carbohydrate 'ligands' which appear on surface glycocomponents of phagocytes (including neutrophils, monocytes and eosinophils), apparently on a subpopulation of skin-homing, memory T-cells and on endothelial cells in high endothelial venules (HEV) [11, 30–32]. Several recent papers provide preliminary structural identifications for the specific complex oligosaccharide ligand(s) which are bound by:

1. LECAM-1 which is also known as LAM-1/Leu-8 ($gp140^{mel}$ in the mouse) and is synthesized and expressed constitutively on lymphocytes, neutrophils and monocytes [33, 34].
2. LECAM-2 which is also known as ELAM-1 and is synthesized and expressed on the surfaces of endothelial cells within 2–4 h of activation by endotoxins, IL-1β, TNFα, substance P, etc. [35–37].
3. LECAM-3 which is also known as GMP-140/PADGEM/CD62 and is carried inside platelet granules and Weibel–Palade bodies of endothelial cells until it is expressed on their surfaces within minutes after activation by thrombin, histamine, oxygen radicals, etc. [38–41].

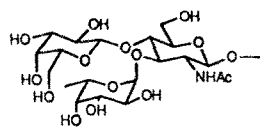
Interestingly, while LECAMs 2 and 3 are up-regulated by the cell activation signals listed above, LECAM-1, which serves nominally as a leukocyte homing receptor [42], appears to be down-regulated in response to inflammatory mediators which activate neutrophils *in vitro* [43]. This suggests a mechanism for dumping leukocytes residing in lymph nodes and Peyer's patches into the systemic circulation. In fact, unique compartmentalization of CAMs seems to be a general feature since, for example, the LEC-CAMs appear to exist preferentially in post-capillary venules, not on arterioles.

Although the 'minimal' oligosaccharide ligand bound by LECAM-1 has not been as clearly defined, it may have the same basic core or backbone structure as that which is bound by the other two selectins; namely, lacto-N-fucopentaose-III (LNF-III). This biologically-important trisaccharide (Fig. 1A) was the first carbohydrate differentiation antigen to be characterized and, in the mouse, is so transiently expressed during embryogenesis that it is called a stage-specific embryonic antigen [44]. In the human, this same structure is designated as the X-hapten of one of the Lewis family of blood group antigens; thus the name, Le^x. In leukocyte nomenclature this antigen system is also designated as 'Cluster of Differentiation 15 (CD15)', and while Le^x is widely distributed in human body tissues and, probably, on circulating macromolecules, among peripheral blood cells it is virtually confined to granulocytes [45].

Addition of a terminal 2,3-linked *N*-acetylneuraminic acid results in Sialyl-Lewis X (SLe^x; Fig. 1B) which constitutes the minimal ligand for LECAM-2 [46–48] and LECAM-3 [49]. Sialic acid has also been shown to be an essential component of the ligand for the homing receptor, LECAM-1 [50]. The majority of investigators studying the minimal structure(s) recognized by LECAM-2 discovered SLe^x. However, one group [51] has identified another ligand; namely, an LNF-III variant which, while still having a terminal sialic acid, has the LNF-III structure separated from sialic acid by a lactosaminyl moiety (Fig. 1C). This structure, recognized by the antibody VIM-2, also occurs on granulocytes [52]. The presence of the two different sialylated LNF-III-type structures on neutrophil surfaces

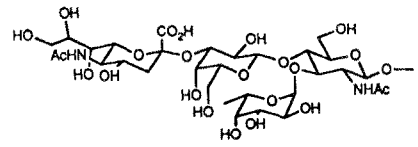
A. LNF-III or Lex

Galβ(1-4)[Fucα(1-3)]GlcNAcβ-



B. Sialyl-Lex or SLex

NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ-



C. VIM-2 antigen or SY-2

NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ-

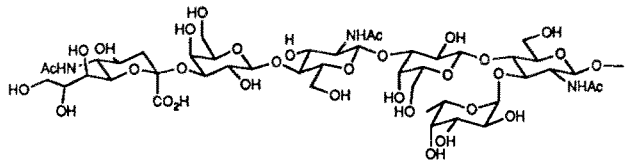


Figure 1. Complex carbohydrate ligands of the vascular selectins.

(SLe^x and the VIM-2 antigen) is probably related to the fine specificities of the unique fucosyltransferase(s) involved.

It is possible that in the future ligand fine structural features (e.g. other carbohydrates, sulphate groups, etc.) will be discovered which identify separate specificities (and biological activities) among the selectins. Thus, as with antibodies to blood group antigens, exquisite specificity may be imparted by a selectin's ability to recognize one or two carbohydrate or sulphated carbohydrate groups in addition to the minimal ligand(s) on leukocyte subsets and/or on HEV cells. This possibility is under intense investigation. Alternatively it is possible that the ligand(s) exist differentially on leukocyte and/or endothelial cell glycoproteins or glycolipids. Clearly, such restriction to one or the other type of cell surface glycoconjugate would suggest different modes of signal transduction and add, indirectly, to arguments for biological specificity residing, in part, in a selectin's ligand.

It is also possible that all three selectins cross-react with cells or macromolecules which have SLe^x-like structures in sufficient concentration on their surfaces, i.e. the selectins may be promiscuous. This is the case for soluble animal lectins such as the serum mannan-binding proteins and conglutinin which cross-react freely with N-linked glycoconjugates which contain Le^a, Le^x, terminal *N*-acetylglucosamine and polymannosyl structures [53, 54]. It is of interest that ligand binding by the soluble animal lectins, which also contain structural motifs analogous to C-binding regulatory proteins, induces quite different biological activities.

There are at least four characteristics of the selectins which would predict different *in vivo* biological activities independent of their carbohydrate ligand(s). First, while similar, their primary structures range in the number of C-binding domain repeats from 2 for LECAM-1 to 9 for LECAM-3. Similarly, since the EGF domain is separated from the cell surface by C-repeats, the EGF domain is more or less 'exposed'. These differences would suggest different affinities for C components and EGF receptors. In support of this hypothesis is some data suggesting that the EGF domain of LECAM-1 may participate in its recognition of lymph node but not Peyer's patch HEVs [55].

Secondly, selectins are found on different cell types. This clearly suggests different biological functions. Thirdly, the selectins are expressed under the influence of a wide variety of cell activation signals. The diversity of signal molecules ranges from complex exogenous agents such as lipopolysaccharide to endogenous bioactive molecules involved in cytokine and thrombogenic or thrombolytic networks to low molecular weight, pharmacological mediators. This diversity suggests (again) that selectins have different intracellular signal transduction mechanisms, and it provides clues as to potential differences in their regional expression. For example, thrombin, histamine and substance P act over short distances *in vivo* – often in selected organs, whereas it is not unusual to find total systemic levels of active IL-1 or TNF.

Lastly, *in vitro* studies suggest that selectins may appear at different times during inflammatory responses and, probably, in varying amounts in different inflammatory conditions. For example, the 'disappearance' of LECAM-2 within 6 h after its up-regulation by IL-1 on human umbilical cord endothelial cells (HUVECs) *in vitro* [56] may be artefactual when considering the more 'chronic' *in vivo* setting. The time-dependent appearance of the selectin however, probably does reflect differences in the relative roles of the various selectins in different inflammatory states. This hypothesis is strengthened by recent findings of LECAM-2 expression in septic but not traumatic shock in the baboon [57]. In fact, the general phenomenon wherein a differential appearance of first, neutrophils and then monocytes in inflamed tissues is observed may now be explained at the CAM level.

Such time-dependent expression would also suggest that the selectins have evolved to assure leukocyte recruitment as a fundamental mechanism in immunoregulation. Consequently, it would not be surprising to find that selectins are highly conserved through evolution. Recognition of this pivotal role for selectins in homeostasis, coupled with the discovery that both antibodies and SLe^x-containing liposomes can block phagocyte adhesion to HUVECs in culture [46], has prompted interest in designing reagents which inhibit selectin–ligand interactions not only as therapeutics, but as a means to discovering the biological significance of the LEC-CAMs.

Assuming a cause and effect relationship, three approaches to blocking selectin–ligand interactions are of interest here. The first is the use of anti-selectin antibodies to, perhaps, capitalize on both the time-course of LEC-CAM expression and on the proposed differential-expression as a function of activation signals. Such reagents are currently being tested in experimental animal models and early results are as provocative as those obtained with antibodies to the integrin and Ig superfamily CAMs [18–20].

The problem with targeting any single CAM is that leukocyte trafficking involves a complex series of events which include initial margination or rolling – which appears to be selectin-mediated [59] – firm adhesion, transmigration through endothelial linings and migration through perivascular connective tissue into the extravascular tissue. At any stage in this sequential process several receptor–ligand pairing interactions may be simultaneously involved [60]. Thus, the adhesion–migration process is probably both multi-component and redundant at the CAM level. Some adhesion molecules may be involved in the entire process, some may be involved with only one event. Selectins are probably involved in the early stages of this scenario when cells moving through the vasculature at more than 2000 $\mu m\ s^{-1}$ need to be slowed and stopped. While there may be a predominant expression of one selectin in a particular inflammatory state, it is not likely that a single selectin will be the sole player in that inflammatory response. Differences between the process itself in different tissues will likely be reflected by subtle variations of the general theme outlined above. However the subtle variations will be of critical importance to the development of useful therapeutic reagents.

It is also unlikely that any selectin will be expressed during only one time-period of a complete inflammatory response. The Yin and Yang of immunology can be expected at the CAM level, and multiple, cooperative down-regulatory events can be expected as the inflammatory response progresses. For example, the appearance of the ligand (SLe^x) on other adhesion molecules, the enhancement and prolongation of LECAM-2 expression by IFN_γ [61] or the persistent presence of SLe^x on cells treated with cytokines which no longer can bind endothelium (J. Harlan, personal communication) are observations which do not fit into simple teleologic concepts developed from *in vitro* studies to date. Thus, the view that blocking one adhesion molecule pair will provide meaningful biological effects is clearly an oversimplification, and the redundancy of CAM interactions suggests that highly specific reagents, such as anti-selectin antibodies, will probably be efficacious only in limited subpopulations of patients – as was seen with single cytokine therapies. As with cytokines, reagents targeting single players in any chronic host response will probably show optimal efficacy in early-stages of that chronic (inflammatory) disease or in combination with other therapies [62].

Consequently, a second approach based on ligand cross-reactivity may be more broadly applicable. This approach would allow therapeutic intervention throughout the time-course of the inflammatory response. In fact, following the assumption that blocking initial rolling or adhesion events will block or suppress subsequent transcellular migration and tissue damage SLe^x-based therapeutics may well be the next generation of non-steroidal anti-inflammatory drugs (NSAIDS). Such reagents will be less toxic also and will provide a level of target selectivity not currently enjoyed by NSAIDS.

While there is hope that low molecular weight, orally-active ligand mimics can be discovered, designers for selectin-based therapeutics should keep in mind the potential importance of ligand multivalence and conformation. It is likely that, as with the hepatocyte membrane Gal/GalNAc receptor [63], 'avidity' or polyvalency amplification of binding will be essential to selectin function. Thus, through the use of multiple linkages (and perhaps by virtue of multiple signals and controls at the level of subcellular organelles) selectins will be found to make up for what will probably be their relatively weak binding affinity(s) and their ligand cross-reactivity(s).

Finally, it is appropriate here to consider anti-carbohydrate (anti-ligand) antibodies or soluble LEC-CAMs as potential therapeutics. Such approaches may be possible if, as with monoclonal antibodies directed against other leukocyte adhesion molecules, the anti-ligand reagents act to 'fine-tune' or down-regulate other leukocyte or endothelial cell functions [64]. However, anti-ligand approaches may also have profound delivery problems since the ligands are found expressed on normal tissue cells (such as hepatocytes) as well as on serum glycoconjugates which may require the SLe^x-like signals to facilitate their trafficking and compartmentalization. Irrespective of whether selectin ligands have such biological (rather than simply physicochemical) importance to non-leukocyte glycoconjugates, their prevalence would necessitate large doses of anti-ligand antibodies to be effective in inflammation.

The major concerns with blocking leukocyte–endothelial cell adhesion in general are the potential deleterious effects of interfering with what appears to be a crucial homeostatic processes. For the selectins in particular, their role in homeostasis remains to be elucidated *in vivo*, but another family of CAMs, the integrins, gave some cause for concern with the 'adhesion-blockade' approach to inflammation. It was found that patients homozygous for a deficiency in synthesis of the integrin B-chain, CD-18 molecule, have leukocyte–adhesion–deficiency (LAD) syndrome whereby they are prone to recurrent and persistent infections [65]. Thus, the question arose as to whether blocking leukocyte adhesion (especially for any long period of time) might not create LAD-like toxicities?

It is important to remember that therapeutics can be used to only partially-block inflammation. This point is exemplified by the LAD heterozygote who has what appears

to be a perfectly normal, protective inflammatory response [65]. Furthermore, transient inhibition of neutrophils with an anti-CD18 antibody (which has profound affects in animal models of ischemia [18–20], does not increase human mortality in abdominal sepsis [66]. Finally, other anti-inflammatory agents such as cyclo-oxygenase inhibitors and conventional NSAIDs are used safely – even chronically. Thus, anti-selectin antibodies and carbohydrate-based therapeutics can be predicted to have good therapeutic ratios with low intrinsic toxicity by virtue of being adaptable to 'tuning' dose, route and schedule parameters.

Of course all of these 'selectin speculations' await experimental testing in animal models of human diseases. What is already clear is that the future holds bright promises for a new wave of approaches to improving human health by capitalizing on the fundamental observation made nearly two decades ago by Ashwell *et al.* [63]; namely, that complex carbohydrates are very important players in cell and molecular social behaviour.

References

1. Taylor FB, Esmon CT, Hinshaw LB (1990) *J Trauma* **30**:197–203.
2. Cotran RS, Gimbrone MA, Jr, Bevilacqua MP, Mendrick DL, Pober JS (1986) *J Exp Med* **164**:661.
3. Warren JS, Kunkel SL, Johnson KJ, Ward PA (1987) *Amer J Pathol* **1129**:578–88.
4. Leung DYM, Pober JS, Cotran RS (1991) *J Clin Invest* **87**:1805–9.
5. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R (1990) *Science* **247**:456–9.
6. Cochrane CG, Janoff A (1974) In *The Inflammatory Process* (Zeifach BW, Rang L, McCluskey RT, eds) p 85. New York: Academic Press.
7. Beck G, Habicht GS, Benach JL, Miller F (1986) *J Immunol* **136**:3025–31.
8. Eddy A, McCulloch LM, Adams J (1990) *J Clin Immunol Immunopath* **57**:441–58.
9. Firestein GS, Zvaifler NJ (1987) *Rheum Dis Clin North Am* **13**:447.
10. Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibovich SJ (1991) *Lab Invest* **64**:313–20.
11. Kyan-Aung U, Haskard DO, Poston RN, Thornhill MH, Lee TH (1991) *J Immunol* **146**:521–8.
12. Norris P, Poston RN, Thomas D, Thronhill M, Hawk J, Haskard DA (1991) *J Invest Dermatol* **96**:763–70.
13. Raine CS, Lee SC, Scheinberg LC, Duijvestin AM, Cross AH (1990) *Clin Immunol Immunopathol* **57**:173–87.
14. Cannella B, Cross AH, Raine CS (1990) *J Exp Med* **172**:1521–4.
15. Malizia G, Calabrese A, Cottone M, Raimondo M, Trejdosiewicz K, Smart C, Oliva L, Pagliaro L (1991) *Gastroenterol* **100**:150–9.
16. Cybulsky MI, Gimbrone MA, Jr (1991) *Science* **251**:788–91.
17. Palabrica TM, Furie BM, Konstam MA, Arnovitz MJ, Connolly R, Brockway BA, Ramberg KL, Furie B (1989) *Proc Natl Acad Sci (USA)* **86**:1036–40.
18. Vedder NB, Winn RK, Rice CL, Chi EY, Arfors KE, Harlan JM (1990) *Proc Natl Acad Sci (USA)* **87**:2643–6.
19. Harlan J (1990) *Surgery* **108**:206–12.
20. Cosimi A, Conti D, Delmonico FL, Preffer FI, Wee S-L, Rothlein R, Faanes R, Colvin RB (1990) *J Immunol* **145**:4604–12.
21. Rice GE, Gimbrone MA, Jr, Bevilacqua MP (1988) *Am J Pathol* **133**:204–10.
22. Humphries MJ, Yamada KM, Olden K (1988) *J Clin Invest* **81**:782–90.
23. Jalkanen S, Aho R, Kallajoki M, Ekfors T, Martamo P, Grahnberg C, Duijvestijn A, Kalimo H (1989) *Int J Cancer* **44**:777–82.
24. Patel K, Bourne S, Phimister B, Coakham H, Kemshead JT (1990) *Biochem Soc Transac* **18**:408–10.
25. Selinka HC, Zilbert A, Wimmer E (1991) *Proc Natl Acad Sci (USA)* **8**:3598–602.
26. Giranda VL, Chapman MS, Rossmann MG (1990) *Proteins* **7**:227–33.
27. Isberg RR (1991) *Science* **252**:934–58.
28. Grau GE, Bieler G, Pointaire P, Heremans H, Dijkmans R, Billiau A (1990) *Immunol Lett* **25**:189–94.
29. Springer TA (1990) *Nature* **346**:425–33.
30. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC (1991) *Nature* **349**:796–8.
31. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, Wagner DD, Furie BC (1989) *Cell* **59**:305–12.
32. Shimizu Y, Shaw S, Graber N, Gopan TV, Horgan KJ, Van Seventer GA, Newman W (1991) *Nature* **349**:796.
33. Jutilla MA, Rott L, Berg EL, Butcher EC (1989) *J Immunol* **143**:3318–24.
34. Watson SR, Fennie C, Lasky LA (1991) *Nature* **349**: 164–7.
35. Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B (1989) *Science* **243**:1160–5.
36. Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers, Gimbrone MA, Jr (1986) *J Immunol* **136**:1680–7.
37. Matis WL, Lavker RM, Murphy GF (1990) *J Invest Dermatol* **22**:492–6.
38. McEver RP, Beckstead JH, Moore KL, Marshal-Carlson L, Bainton D (1989) *J Clin Invest* **84**:92–9.
39. Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA, McEver RP (1990) *Nature* **343**:757–60.
40. Patel KD, Zimmerman GA, Prescott SM, McEver RP, McIntyre TM (1991) *J Cell Biol* **112**:749–59.
41. Toothill VJ, Van Mourik JA, Niewenhaus HK, Metzlaar MJ, Pearson JD (1990) *J Immunol* **145**:283–91.
42. Gallatin WM, Weissman IL, Butcher EC (1983) *Nature* **304**:30–4.
43. Kishimoto TK, Jutilla MA, Butcher EC (1990) *Proc Natl Acad Sci (USA)* **87**:2244–8.
44. Gooi HC, Feizi T, Kapadia A, Knowles BB, Solter D, Evans MJ (1981) *Nature* **292**:156–8.
45. Hakomori S, Kobata A (1974) In *The Antigens* (Sela M, ed.) p 79–140. New York: Academic Press.
46. Phillips ML, Nuddleman E, Gaeta FC, Perez M, Singhal AK, Hakomori S, Paulson JC (1990) *Science* **250**:1130–2.

47. Walz G, Aruffo A, Kolanus W, Bevilacqua MP, Seed B (1990) *Science* **250**:1132–5.
48. Lowe JB, Stoolman LM, Nair RP, Larsen RD, Berhend TL, Marks RM (1990) *Cell* **63**:475–84.
49. Polley MJ, Phillips ML, Wayner E, Nudelman E, Singhal AK, Hakomori S, Paulson JC (1991) *Proc Natl Acad Sci (USA)* **88**:6224–8.
50. True DD, Singer MS, Lasky LA, Rosen SD (1990) *J Cell Biol* **111**:2757–64.
51. Tiemeyer M, Swiedler SJ, Ishihara M, Moreland M, Schweingruber H, Hirtzer P, Brandley BK (1990) *Proc Natl Acad Sci (USA)* **88**:1138–42.
52. Macher BA, Buehler J, Scudder P, Knapp W, Feizi T (1988) *J Biol Chem* **263**:10186–91.
53. Childs RA, Drickamer K, Kawasaki T, Thiel S, Musuochi T, Feizi T (1989) *Biochem J* **262**:131–8.
54. Drickamer K (1988) *J Biol Chem* **263**:9557–60.
55. Siegelman MH, Cheng IC, Weissman IL, Wakeland EK (1990) *Cell* **61**:611–22.
56. Lo SK, Detmers PA, Levin SM, Wright SD (1989) *J Exp Med* **169**:1779–93.
57. Redl H, Dinges HP, Buurman WA, van der Linden CJ, Pober JS, Cotran RS, Schlag G (1991) *Amer J Pathol* (in press).
58. Stoolman LM (1989) *Cell* **56**:907–10.
59. von Andrian UH, Chambers JD, McEvoy LM, Bargatze RF, Arfors KE, Butcher EC (1991) *Proc Natl Acad Sci (USA)* (In press).
60. Luscinskas FW, Cybulsky MI, Kiely J-M, Peckins CS, Davis VM, Gimbrone MA, Jr (1991) *J Immunol* **146**:1617–25.
61. Leeuwenberg JFM, Von Asmuth EJU, Jeunhomme TMA, Buurman WA (1990) *J Immunol* **145**:2110–4.
62. Winkelhake JL, Gauny SG (1990) *Pharmacol Rev* **42**:1–28.
63. Ashwell G, Harford J (1982) *Ann Rev Biochem* **51**:531–54.
64. Geissler D, Gaggli S, Most J, Greil R, Herod M, Dierich M (1990) *Eur J Immunol* **20**:2591–6.
65. Anderson DC, Springer TA (1987) *Ann Rev Med* **38**: 175–9.
66. Mileski WJ, Winn RK, Harlan JM, Rice CL (1991) *Surgery* **109**:497–501.

Letters or comments relating to this article would be received with interest by Pauline Rudd, Assistant to the Special Advisory Editor, R. A. Dwek.