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REVIEW ARTICLE

# Carbohydrate-Dependent Cell Adhesion

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## 1. Introduction

Cell surface carbohydrates are major components of the outer surface of mammalian cells and are very characteristic of cell types. In the past few years, there has been a surge of new data revealing the roles of one of the cell-type specific carbohydrates, sialyl Lewis x (sialyl Le$^x$). Sialyl Le$^x$, specifically expressed on granulocytes and monocytes, were found to be recognized by cell adhesion molecules present on platelets and endothelia cells. This discovery has brought about an entirely new era in glycobiology, which addresses the functions of carbohydrates in cell recognition. In this short review, I would like first to summarize the knowledge on cell-type specific carbohydrates, focusing those present on human blood cells (section 2). I will then provide a summary on animal lectins, in particular the presence of carbohydrate recognition domain in these lectins (section 3). Those critical findings have made it possible to identify a new class of adhesive molecules, selectins (section 4). As soon as selectins were found to have carbohydrate binding properties, the ligand, sialyl Le$^x$, was identified, mainly because this structure had been determined in the previous studies as cell-type specific carbohydrates (section 5). Currently, extensive studies are being carried out to understand the roles of carbohydrates in inflammation and to search for carbohydrate-based therapeutic drugs (section 6). In addition, the work has been carried out to determine if tumor cells utilize a similar mechanism for metastatic dissemination (section 7).

## 2. Cell-Type Specific Glycosylation

Carbohydrates are major components of the outer surface of animal cells. A characteristic feature of these carbohydrates is that they show a tremendous heterogeneity. However, it was gradually recognized that these carbohydrates are very often characteristic of cell types. Thus, the presence of specific sets of carbohydrates are frequently restricted to certain cell lineages or certain stages of differentiation in a given cell lineage. In order to determine how different cell-types express different carbohydrates, it has been useful to work on carbohydrates present in blood cells.[1] In particular, it was of significance to determine the carbohydrate structures present on erythrocytes and granulocytes, since both cells are directly derived from common precursor cells yet differ tremendously in their functions.[1,2] Human erythrocytes are characterized by having a large amount of ABO blood group antigens. This class of antigens was shown to be carried by poly-*N*-acetyllactosamines, which are present mainly on *N*-glycans and glycolipids. In later studies, it has been demonstrated that ABO blood group antigens reside mainly in *N*-glycans contributing to the majority of the antigens in cells,[3–5] although earlier studies demonstrated the presence of ABO blood group antigens in glycolipids.[6]

In contrast to erythrocytes, granulocytes do not express ABO blood group antigens. Granulocytes (and monocytes) synthesize instead a large amount of Le$^x$ structure, which is an isomer of Lewis blood group a and b (Fig. 1). Some of the side chains contain additional sialic acid, thus forming sialyl Le$^x$ structure.

The formation of these two different fucosylated structures, ABO and sialyl Le$^x$, are due to two entirely different fucosyltransferases. While H antigen, a precursor of blood group A and B antigens, is formed by

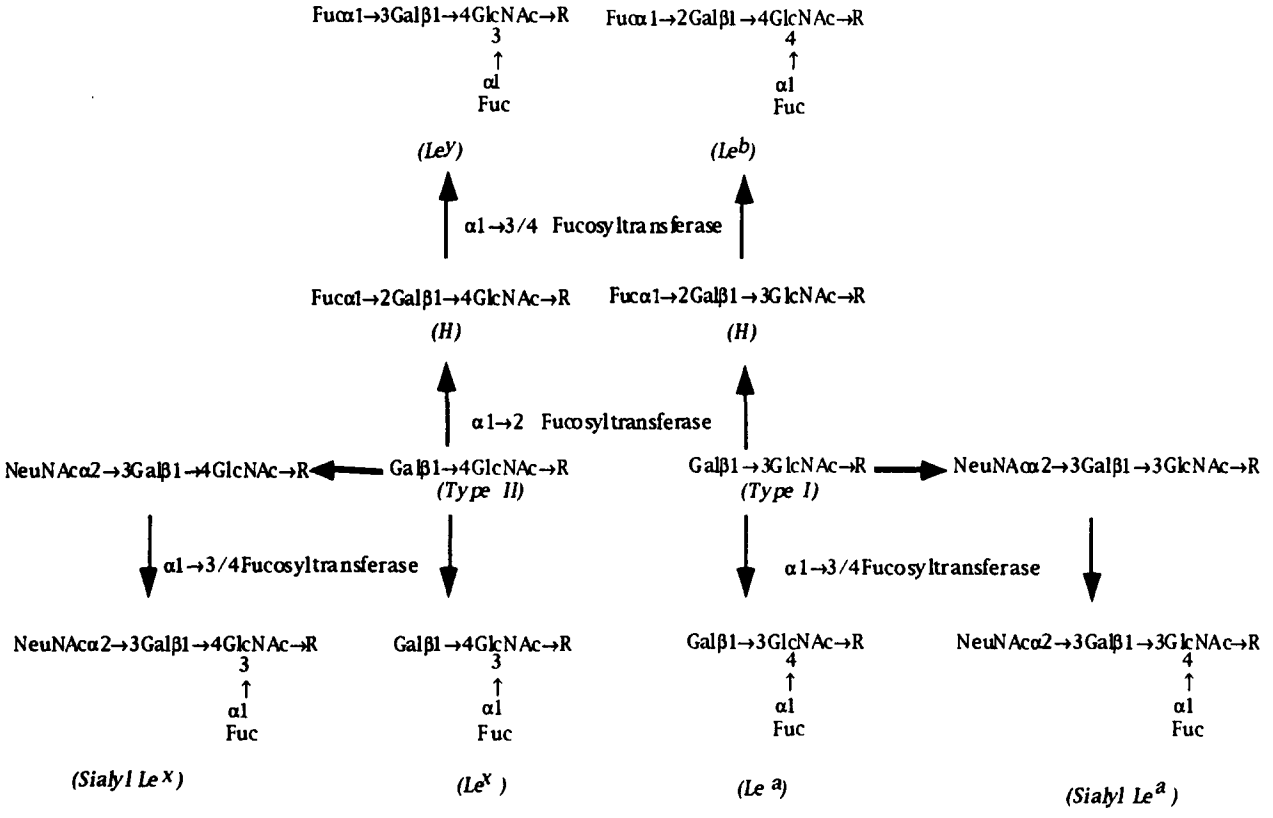


**Figure 1.** Structure and biosynthesis of Lewis and Lewis-related oligosaccharide determinants. $Le^a$ and $Le^b$ constitute a part of Lewis blood group antigens, while $Le^x$ constitutes a differentiation antigen. Blood cells express only type II chains while other tissues such as gastrointestinal duct express type I chains as well. Type II oligosaccharides in blood cells are fucosylated by α1→3 fucosyltransferase, while type I and type II oligosaccharides in gastrointestinal tissues are mostly fucosylated by Lewis enzyme, α1→3/4 fucosyltransferase.

H forming α-1,2-fucosyltransferases, $Le^x$ antigen is formed by α-1,3-fucosyltransferase (Fig. 1). Most recent studies revealed that there is only one α-1,2-fucosyltransferase which is responsible for H structure formation in blood cells.[7] On the other hand, it was discovered recently that the formation of $Le^x$ and sialyl $Le^x$ in leukocytes are catalyzed by two distinct fucosyltransferases, IV and VII, respectively.[8,9] All other α-1,3-fucosyltransferases present in other tissues catalyze the formation of both $Le^x$ and sialyl $Le^x$, and they do not show any specificities for the formation $Le^x$ or sialyl $Le^x$. Fucosyltransferase III, present in epithelial cells, even transfers fucose residues to different acceptors, type-1 based Galβ1→3GlcNAc and type 2 based Galβ1→4GlcNAc structures, forming $Lewis^a$ and $Lewis^x$ antigen, respectively.[10] These results indicate that the formation of sialyl $Le^x$ and $Le^x$ in leukocytes is more strictly regulated than in other tissues. I will discuss the significance of these oligosaccharides in later sections.

## 3. Animal Lectins—Carbohydrate Recognition Domain

It has been demonstrated from the early 1950s that there is a group of plant proteins which agglutinate red cells. These proteins present in plant seeds were found to bind to the carbohydrates on the surface of red cells and were eventually called "lectins". The discovery of animal lectins was made from an entirely different line of studies. In the early 1970s, Ashwell and Morell found that desialylated plasma glycoproteins were cleared much faster than intact glycoproteins from circulation. They found that desialylated glycoproteins cleared from circulation were accumulated in the liver, in particular hepatocytes. This endocytic uptake was then found to be due to a galactose-binding lectin, which binds terminal galactose residues, exposed by removal of sialic acid.[11] Further, studies revealed that there are probably more than a dozen carbohydrate binding proteins in the animal kingdom and they are collectively called animal lectins. The amino acid sequences of these animal lectins revealed that there are at least two different kinds of animal lectins: one is functional in the presence of calcium ion and the other in the presence of sulfhydryl groups.[12] The former is called C-type lectin and the latter S-type lectin. In other aspects these two types of lectins differ significantly. A good proportion of C-type lectins, including the galactose-binding protein, are membrane glycoproteins. These C-type lectins are expressed in a more tissue-specific manner than S-type lectins. In addition, researchers have identified biological functions for C-type lectins, such as endocitosis receptors, IgE Fc receptor and a pulmonary surfactant, while little is known about biological functions of S-type lectins.

Comparison between the amino acid sequences of these C-type lectins revealed that there is a conserved amino acid sequence or motif which includes two disulfide-bonded loops and conserved amino acid residues. Since these amino acid residues are likely to form a common framework it is called carbohydrate recognition domain (CRD).[12] Conserved amino acid sequences are also present in different S-type lectins, called S-type CRD. Recently, the crystallographic studies revealed the three-dimensional structure of rat mannose-binding protein, one of the C-type lectins. The results indicate that CRD forms a framework for the binding of a mannose and its formation is clearly dependent on the presence of two $Ca^{2+}$ ions.[13] The $Ca^{2+}$ ion in this mannose binding protein also ligates the 3-OH and 4-OH in the mannose ring. Since the 2- and 3-OH groups of fucose can be superimposed on the 3- and 4-OHs of mannose, the $Ca^{2+}$ ion can most likely coordinate the 2- and 3-OH groups of fucose which is a part of the carbohydrate ligand, sialyl $Le^x$ (see the following section).

Each complex coordinated by $Ca^{2+}$ is then stabilized by hydrogen bonds between sugars and proteins. One critical discovery from this three-dimensional structure is the presence of two glutamic acid–asparagine pairs which are required for the binding to mannose residues. An elegant study was then made by Drickamer.[14] He demonstrated that the replacement of one of the pairs (Glu185 and Asn187) with that of the galactose binding protein (Gln185 and Asp187) converted this mannose binding protein to a galactose binding protein. These results clearly indicate that there are a few amino acid residues which are critically involved in carbohydrate recognition.

Selectins are a group of cell surface proteins which most recently joined the group of C-type lectins. Selectins are adhesive proteins present in platelets, endothelial cells or leukocytes[15] and their deduced amino acid sequences revealed that CRD is present in the $NH_2$-terminal domain of these selectins (Fig. 2). Selectins are unique in that they bind to relatively restricted types of carbohydrate structures, a property which probably ensures a specific binding in cell–cell interactions. For example, E- and P-selectin bind the sialyl $Le^x$ structure much more efficiently than the$Le^x$ structure.

Most recent crystallographic studies revealed the three-dimensional structure of the lectin-like and EGF domains of E-selectin.[16] The results showed that the lectin-like domain or CRD of E-selectin contains only one $Ca^{2+}$ ion. The results also suggested that this $Ca^{2+}$ is posing to bind to the 2- and 3-OH groups of fucose. Although this suggestion is discerned from the comparison with the structure of the mannose-binding protein,[13] the presumed structure is likely the case since sialyl $Le^x$ in the complex fits well with the sialyl $Le^x$ structure determined by NMR. Similar to the mannose-binding protein, Glu80, Asn82 and Asn105 and Asn106 of E-selectin are involved in $Ca^{2+}$ binding. However, Glu88 is not involved in the binding of $Ca^{2+}$, although the corresponding Glu193 in the mannose-binding protein is involved. Apparently selectins are unique in having only one bound calcium ion.

The other important aspect in this three-dimensional structure is that the lectin-like domain and EGF-like domain are not close to each other. This leads to the prediction that the majority of carbohydrate binding specificity is determined by the structure of the lectin-like domain and may not be influenced by the EGF-like domain.

In contrast to accumulated knowledge on biological functions of C-type lectins, there is only limited information about the functions of S-type lectins. A part of this problem stems from the fact that these lectins are widely distributed in various tissues with no clear tissue-specificity in their expression. The majority of S-type lectins most efficiently bind galactose and *N*-acetyllactosamine and lack a rigid specificity in binding to carbohydrates. According to the molecular weights of S-type lectins, they are roughly grouped into L-14 (14 kDa) and L-30 (30 kDa) lectins. There is, however, a subtle preference for certain sugars which are bound by one of these proteins, for example, L-14 binds more strongly to Galβ1→4GlcNAc than galactose and binds efficiently to poly-*N*-acetyllactosamine.[17] More recently, crystal structures of 14 kDa bovine spleen lectin and human dimeric S-Lac lectin were elucidated.[18,19] Both studies revealed that the $N^{\eta}$ atom of Arg48 and the $N^{\varepsilon}$ atom of His44 form two key electrostatic interactions with the axial 4-OH of galactose, a main determinant of the S-lectin specificity.[18,19] In glucose and *N*-acetylglucosamine, the

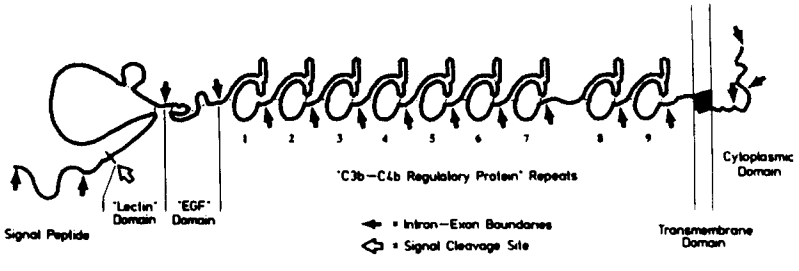


**Figure 2.** Domain structure of P-selectin. The open arrow indicates the site for proteolytic removal of the signal peptide. Cysteine residues are indicated by filled circles, while the other amino acid residues are shown by circles. Each domain is coded by different exons and intron–exon boundaries are indicated by closed arrows. This Figure was taken from reference 35.

4-OH is equatorial, distinguishing the galactose from the other sugars. The N2 atom of the *N*-acetyl group in *N*-acetyllactosamine is involved in a water-mediated interaction with the side chains of Arg93 and Asp54 with the main chain carbonyl of His52. The binding of *N*-acetyllactosamine to S-lectin is about 5-fold higher than the binding of lactose, probably because of van der Waals interactions between the *N*-acetyl group and the side chains of Arg73 and Glu71 in the former interaction.[18]

The third group of animal lectins is the mannose-6-phosphate receptors which recognize mannose-6-phosphate residues present in high-mannose oligosaccharides of lysosomal enzymes. This recognition is highly specific and only high-mannose oligosaccharides containing 6-phosphate groups are recognized. Such a highly specific recognition ensures that only lysosomal enzymes are successfully transported to lysosomes. The binding of the receptors to mannose-6-phosphate is efficient only when two binding sites of a lysosomal enzyme are recognized by two receptors in the case of a 45 kDa receptor, the cation-dependent receptor, or by coordination of two repeating units in the large 290 kDa cation-dependent receptor.[20] These results reinforce the idea that a sufficient binding between carbohydrate and protein can be achieved by multimeric interactions, mainly because monomeric binding is not a high affinity interaction. At the same time, this allows a quick dissociation between two molecules when they need to be separated. For example, the mannose-6-phosphate receptor is dissociated from lysosomal enzymes once the complex reaches prelysosomes, leaving behind the enzymes in prelysosomes, while the receptor recycles back to the Golgi complex. This enables economical usage of the receptors, avoiding *de novo* synthesis after each traffic (see also section 5).

## 4. Oligosaccharides in Lymphocyte Homing

When peripheral lymphocytes are collected, labeled and injected again into the vascular system, these lymphocytes are concentrated in lymph nodes and other lymphatic organs. This phenomenon is called "lymphocyte homing", since it appears as though they go back to their original place.[21] This homing is actually a part of a process which allows the lymphocytes in the bloodstream to leave the vascular trees within lymph nodes. These cells then reach the parencyma of the lymph node or other lymphatic organs, pass through the lymphatic system, and return to the vascular system at the thoracic duct, going back to the vein in the vascular system. Since this traffic is heaviest through the organs specialized for concentration and processing of the antigens, the reinjected lymphocytes appear to home in lymph nodes, Peyer's patch, lung, spleen and bone marrow.

Lymphocytes leave the vascular tree during their passage through the postcapillary venules within lymphoid organs. This event involves initial adhesion of lymphocytes on the cell surface of postcapillary venular endothelia. By generating various monoclonal antibodies against lymphoid cells, it was discovered that a certain group of antibodies specifically inhibit the attachment of lymphocytes to postcapillary high endothelial venules (HEV), which are the gates from the vascular system to the lymphoid system. An assay system was established to measure the binding of lymphocytes to HEV exposed in frozen sections of peripheral lymph nodes.[22] By using a monoclonal antibody called MEL-14, this process is efficiently inhibited. The antigen recognized by this antibody is present in all lymphocytes. When lymphocytes are incubated with a saturating amount of MEL-14, *in vivo* homing of lymphocytes to peripheral nodes can be inhibited. The protein recognized by this MEL-14 is called the homing receptor. Pioneer work done by Rosen and Stoolman established that this interaction between the homing receptor and HEV is dependent on carbohydrates. In particular, phosphorylated mannose polymer and fucoidan, sulfated high molecular weight polymers containing $\alpha 1 \rightarrow 2$ and $\alpha 1 \rightarrow 3$ linked fucose residues, were found to be effective inhibitors of this interaction.[23]

The importance of sialic acid was first realized from the fact that lymphocytes no longer adhered to HEV once the latter was treated with sialidase.[24] As a complementary approach, it was demonstrated that phosphorylated mannose polymer can directly bind to the MEL-14 antigen. These results and others established that the MEL-14 antigen is a glycoprotein, which binds a carbohydrate ligand displayed at the surface of HEV in the peripheral lymph nodes.

By using MEL-14 antibody and corresponding antibodies specific to a human counterpart, mouse and human lymphocyte homing receptors were cloned. The deduced amino acid sequence revealed the presence of three distinct structural domains: the $NH_2$-terminal domain contains lectin-like motif having CRD, epidermal growth factor precursor (EGF) is next to it and then two of the complement receptors repeat[25] (see also Fig. 2). The focus of the structure of L-selectin lies in its $NH_2$-terminal lectin-like domain.[25] It is not clear how the other two domains contribute to the function of the lectin-like domain. However, it was reported that the EGF domain apparently modulates the carbohydrate-binding property (but see also above on the three-dimensional structure).[26]

Despite the fact that the lymphocyte homing receptor is the first clear-cut case to show that selectins bind to carbohydrates, the exact structure of the carbohydrate ligand is not known. However, some information has accumulated on the nature of the physiological ligand. First, two glycoproteins were identified in lymph nodes which bind to L-selectin. They are GlyCAM-1[27] and CD34.[28] Both glycoproteins are characterized by their high content of mucin-type O-glycans. Secondly, L-selectin–carbohydrate interaction is dependent on sialic

acid. Thirdly, it is essential to have sulfate groups in this ligand. When sulfation was inhibited by incubation of the lymphocytes with chlorate, L-selectin no longer binds to glycoproteins containing presumably unsulfated ligands.[29]

There are several reports demonstrating that exogenously added sialyl $Le^x$ can inhibit the interaction between L-selectin and GlyCAM-1 at a high concentration (10 mM).[28] Considering that the fucose polymer also inhibits L-selectin binding to GylCAM-1, sialyl $Le^x$ structure is likely to be included in the physiological ligand. Most recently, sulfated sialyl $Le^x$, NeuNAcα2→3(6-sulfate)Galβ1→4(Fucα1→3)GlcNAc→, was demonstrated in the terminal carbohydrate structures attached to GlyCAM-1.[31] This is a good candidate for the physiological ligand. Both GlyCAM-1 and CD34 are efficient carrier glycoproteins for this ligand, probably because this oligosaccharide is presented by GlyCAM-1 and CD34 as a multimer to L-selectin. It is suggested that L-selectin is present as dimeric or oligomeric forms.

## 5. Oligosaccharides in Inflammation

When inflammation takes place, endothelial cells newly express adhesion molecules which bind to neutrophils, monocytes, eosinophils, memory T-cells and NK-cells. This binding ("rolling effect") allows leukocytes to have enough access to chemokines, which are presented by a heparin sulfate or other proteoglycans.[32] Once chemokines are bound to their receptors or leukocytes, a signal is presumably transmitted through G-proteins, which eventually activates integrins on the surfaces of leukocytes, establishing a firm attachment to endothelia.[15] This firm attachment then leads into extravasation, through homophilic interaction of PECAM-1 (Fig. 3).[33] For this initial step to occur, it was shown that endothelial cells need to be activated by inflammatory agents, such as interleukin-1β, tumor necrosis factor-α, and lipopolysaccharides, in order to induce the adhesive molecule(s). By raising monoclonal antibodies against activated endothelial cells, it was discovered that a protein with $M_r$ ~115,000 is temporally expressed on the surface of endothelial cells, which correlates with the temporal pattern of cytokine dependent adhesion. This protein was initially named endothelial leukocyte adhesion molecule I, or ELAM-1. Later, this was termed E-selectin.

Using the antibody described above, human E-selectin cDNAs were cloned.[34] The deduced amino acid sequence revealed domain structures which are similar to those of L-selectin. The $NH_2$-terminal domain is a lectin-like domain containing C-type lectin CRD. This is followed by an EGF-like domain and four complement repeat domains (see also Fig. 2). These three domains are connected to the transmembrane and cytoplasmic domains. The $NH_2$-terminal domain and EGF-like domain are 61% identical at the amino acid level to the corresponding domains in L-selectin, whereas their transmembrane and cytoplasmic domains are less than 10% homologous. A similar protein, originally discovered in platelet α-granules, was purified and its cDNA was cloned.[35] This protein, now called P-selectin, has very similar domain organizations (Fig. 2).

### *Sialyl $Le^x$ oligosaccharide-ligands for E-selectin*

Since E-selectin was found to contain a lectin-like domain in its $NH_2$-terminus, it was an immediate task to look for carbohydrate ligands that are recognized by this selectin.

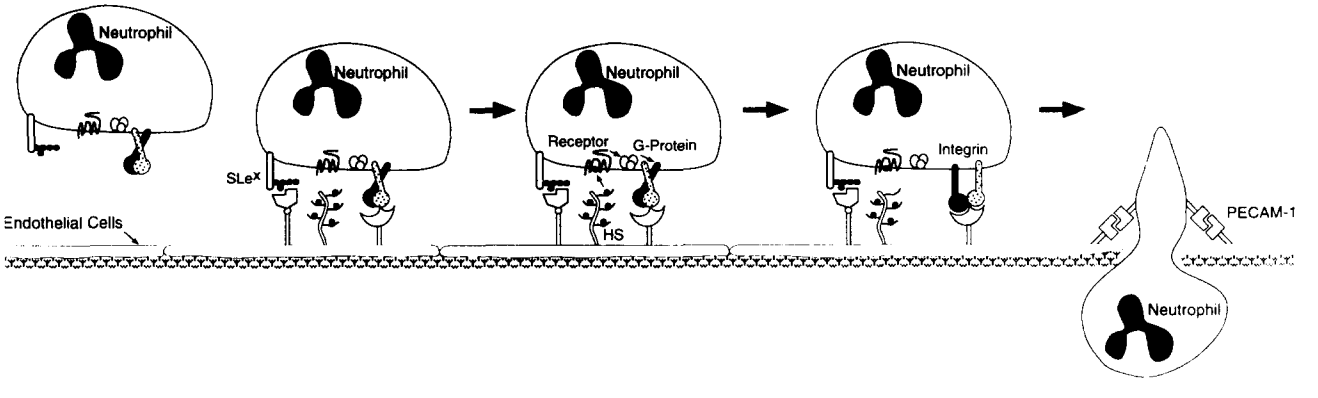


**Figure 3.** Schematic representation of neutrophil and monocyte adhesion to endothelial cells. In unstimulated state, leukocytes are rapidly flowing through the blood vessels. Once endothelial cells are activated by inflammatory agents, E-selectin (and P-selectin) are transported to the cell surface and bind to leukocytes resulting in the slow down leukocytes, or "rolling effect". Once leukocytes are close to endothelial cells because of rolling, chemoattractants such as MIP, originally bound to cell surface heparin sulphate, are transferred to the receptor (which is most likely a member of the seven transmembrane receptors). The chemoattractant then induces signals, probably through G protein, which then induces upregulation or conformational change of integrins. Those active integrins now bind to the ICAM-1 in endothelial cells, establishing tight binding to endothelials. The last step then leads to the penetration of neutrophils between the boundary of endothelial cells most likely through PECAM-1 interaction, resulting in vascular extravasation.

E-selectin binds to the cell surface of myeloid cells and the structures of those carbohydrates on granulocytes were elucidated by our group and others.[36,37] These studies demonstrated that $Le^x$, Galβ1→4(Fucα1→3)-GlcNAcβ1→R, and sialyl $Le^x$, NeuNAcα2→3Galβ–1→4(Fucα1→3)GlcNAcβ1→R are enriched in these cells. It was thus natural that these oligosaccharides were tested for their binding capability to E-selectin. In particular, Lowe *et al.* utilized two different α-1,3-fucosyltransferases to express unique sets of carbohydrates in CHO cells. Fuc-TIII directed the formation of sialyl $Le^x$, $Le^x$ and VIM-2 structure, which is NeuNAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4(Fucα1→3)GlcNAcβ1→R. In contrast, Fuc-TIV directed mainly the formation of $Le^x$ with a small amount of VIM-2 structure.[38,39] When these cells were tested for adhesion to E-selectin, CHO cells with sialyl $Le^x$ adhered well to E-selectin, while those with only $Le^x$ and VIM-2 structure barely adhered.[38,39] Similar results were obtained by other investigators when different glycolipilids were used as inhibitors against E-selectin mediated adhesion of HL-60 cells.[40,41] In addition, sialyl $Le^x$ was found to be a potent inhibitor towards P-selectin mediated adhesion.[42] These results establish that sialyl $Le^x$ is most likely the ligand for E- and P-selectins.

Tumor cells, in particular carcinomas, are enriched with sialyl $Le^a$, NeuNAcα2→3Galβ1→3(Fucα1→4)-GlcNAc→ in addition to sialyl $Le^x$ structures.[43] Since sialyl $Le^a$ is the isomer of sialyl $Le^x$, sialyl $Le^a$ was tested for binding capability to E-selectin. In one study, it was found that sialyl $Le^a$ has a slightly higher affinity for E-selectin than does sialyl $Le^x$.[44] The other studies demonstrated that E-selectin-mediated adhesion of colorectal carcinoma cell line Colo 201 was inhibited by anti-sialyl $Le^a$ antibodies but not by anti-sialyl $Le^x$ antibodies.[45] Colo 201 cells are apparently enriched with sialyl $Le^a$ structure and contain only a small amount of sialyl $Le^x$ structures.

These results establish that E-selectin and possibly P-selectin bind to sialyl $Le^x$ and sialyl $Le^a$ structures depending on cell-type. Although it is obvious that sialyl $Le^x$ and sialyl $Le^a$ are primarily recognized by these selectins, it is not entirely sure that simple presentation of these oligosaccharides support sufficient binding by these selectins. This is more apparent when P-selectin- and E-selectin-mediated adhesion are compared. For example, HL-60 cells were found to express a small number of P-selectin binding sites with high affinity. In contrast, CHO-FTIII cells were found to express a large number of low affinity P-selectin binding sites. More recently, a glycoprotein with ~120,000 kDa, PSGL-1, was cloned by virtue of its support of high affinity binding to P-selectin.[46] This was possible because P-selectin does not bind efficiently to COS cells that express sialyl $Le^x$ and sialyl $Le^a$ structures. The amino acid sequence of PSGL-1 was found to contain a stretch which likely attaches a large number of mucin-type *O*-glycans. As mentioned already, the high affinity ligand for L-selectin is also presented by two mucin-type glycoproteins, CD34 and GlyCAM-1. Taken together, these results strongly suggest that P- and L-selectins bind much more efficiently to carbohydrate ligands that are present in mucin-type *O*-glycans. Since these carrier molecules present the carbohydrate ligands as a cluster, it is possible that multiple presentation of the ligands leads into high affinity binding of P- and L-selectin. In contrast, E-selectin apparently binds to the carbohydrate ligands regardless of how they are presented.

As mentioned above, mannose-6-phosphate receptors bind to two ligands when they achieve physiologically significant bindings. It is thus tempting to conclude that carbohydrate–protein interactions require multiple ligand–receptor bindings in order to achieve a physiologically meaningful interaction. This is probably because this kind of interaction should be dynamic and transient in nature and its dissociation is necessary when there is no need for further association. This is true for both mannose-6-phosphate receptors and selectins (Fig. 3).

It is noteworthy that E-selectin apparently binds to the carbohydrate ligands regardless of the carrier molecules. Although this difference in requirement for ligand presentation between E-selectin and P- or L-selectin is not understood, crystallographic studies on P- or L-selectin hopefully provides insight for understanding the difference in carbohydrate recognition among these molecules. Alternatively, it is possible that P- or L-selectin binds to carbohydrates with physiologically meaningful affinity only when these selectins are dimers or multimers.

## 6. Sialyl $Le^x$ Containing Glycoconjugates as Therapeutic Agents

E-Selectin is expressed by capillary endothelium in a wide variety of acute and chronic inflammatory conditions. These include rheumatoid arthritis, sepsis, immune complex-dependent acute lung injury, delayed hypersensitivity in the skin, and in human cardiac allografts.[47] Carbohydrate ligands or counter-receptor(s) for E-selectin were found on neutrophils, monocytes, eosinophils, memory T-cells and NK cells. Those cells are accumulated at inflammatory sites where E-selectin is expressed.[47] It is thus expected that the carbohydrate–E-selectin interaction plays a critical role in recruitment of these cells to the sites where acute and chronic inflammation takes place.

Once the importance of E-selectin–carbohydrate interaction is recognized, it is possible to develop therapeutic agents which are antagonistic towards unnecessary inflammatory responses. In particular, this has been well documented in acute lung injury of rats, caused by the injection of a toxin or formation of an immune-complex.[48] This acute lung injury is thought to

be a model for acute respiratory syndrome in humans. In this syndrome, the majority of patients reach coma because of the acute inflammatory response in lungs. This symptom is associated with trauma, for example, caused by a burn covering a large portion of the body. Once this trauma is initiated, bronchi are filled with granulocytes, leading to hemorrhages. Often such an acute inflammation leads to the collapse of a lung and the incapacitation of breathing.

Ward and his colleagues demonstrated that the acute lung inflammation was suppressed when P-selectin or anti-E-selectin antibodies were administered at the onset of inflammation.[49] Moreover, it was demonstrated that glycopeptides containing sialyl $Le^x$ or sialyl $Le^x$ oligosaccharides are powerful enough to inhibit the acute inflammatory response.[50] In relation to these studies, our laboratory has shown that recombinant glycoproteins containing sialyl $Le^x$ structures can efficiently inhibit the cell adhesion to colon tumor cells, which is mediated by E-selectin.[51,52] These results clearly indicate that carbohydrate-based therapeutic drugs can be developed.

## 7. Carbohydrate-Mediated Adhesion of Tumor Cells

Tumor cells, in particular leukemia and carcinomas, are enriched with sialyl $Le^x$ and sialyl $Le^a$ structures. When tumor cells disseminate, tumor cells that have entered the blood flow need to adhere to endothelial cells at metastatic sites. Once tumor cells adhere to endothelial cells, they then penetrate through endothelial cells, moving into subendothelial tissues. Once tumor cells grow in beneath endothelial tissue, tumor metastasis is established.[53]

This process is very much reminiscent of the movement of leukocytes during inflammation. It is thus reasonable to assume that similar mechanisms might be working when tumor cells attach to endothelial cells at metastatic sites. In fact, it was shown recently that highly metastatic tumor cells express more sialyl $Le^x$ structures on the cell surface, which are attached to carrier molecules such as lysosomal membrane glycoprotein-1 (lamp-1).[54]

It has also been demonstrated that highly metastatic tumor cells bind more strongly to E-selectin expressed on activated endothelial cells than low metastatic tumor cells. This is true also for mouse-derived endothelial cells.[52] The latter findings indicate that mouse E-selectin binds efficiently to sialyl $Le^x$ structure and metastasis in nude mice mostly reflects tumor metastasis in humans, at least, at the level of E-selectin mediated adhesion.[52] Tumor cells expressing low levels of cell surface lamp-1 were genetically engineered to increase the amount of cell surface lamp-1. Resultant cells were found to adhere more strongly to E-selectin as the cells express more sialyl $Le^x$ on cell surface.[51]

These results were reinforced by the recent discovery that the amount of sialyl $Le^x$ is directly correlated to the prognosis of colonic carcinoma patients. The patients with higher expression of cell surface $Le^x$ structures show much poorer prognosis than those with lower expression of sialyl $Le^x$, although other histological criteria were apparently similar.[55] These combined studies strongly suggest that one of the key factors in metastatic spread is the amount of sialyl $Le^x$ structure, the ligand for E- and P-selectin in tumor cells. Further studies will be of significance if metastatic processes can be inhibited by sialyl $Le^x$-containing glycoproteins or oligosaccharides.

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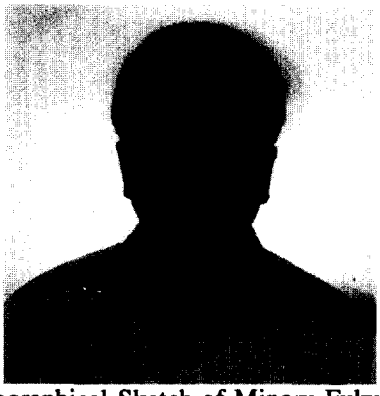

Biographical Sketch of Minoru Fukuda

Minoru Fukuda was born in Taiwan while it was a part of Japan, in 1945, and received a Bachelor of Science degree in Biochemistry from the University of Tokyo in 1968. He received a Ph.D. working with Fujia Egami and Toshiaki Osawa from the University of Tokyo in 1973. After he worked as a research associate for two years at the University of Tokyo, he trained as a postdoctoral associate for two years at the Yale University School of Medicine. In 1977, he then joined the Division of Biochemical Oncology at the Fred Hutchison Cancer Research Center. After he became assistant professor there, he joined the La Jolla Cancer Research Foundation in 1982 where he is now Senior Staff Scientist and Director of the Glycobiology/Chemistry Program. He also has an adjunct professor appointment at the University of California at San Diego. His research interest lies in the biochemistry of cell surface carbohydrates, particularly in the identification of cell-type specific carbohydrates, their roles in cell–cell interaction, isolation of glycosyltransferase genes and elucidation of the roles of carbohydrates by the expression of glycosyltransferase genes.