Chapter 31 – C-type Lectins

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Essentials of Glycobiology 2nd edition
Chapter 31

C-type Lectins

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C-type lectins are Ca\(^{2+}\)-dependent glycan-binding proteins that share primary and secondary structural homology in their carbohydrate-recognition domains (CRDs). These proteins have a C-type lectin fold, which is a fold with highly variable protein sequence that is also present in many proteins that do not bind carbohydrates (C-type lectin domain [CTLD]-containing proteins). C-type lectins and proteins with CTLDs are found in all organisms. The large family of C-type lectins includes collectins, selectins, endocytic receptors, and proteoglycans. Some of these proteins are secreted and others are transmembrane proteins. They often oligomerize into homodimers, homotrimmers, and higher-ordered oligomers, which increases their avidity for multivalent ligands. Although they share structural homology, C-type lectins usually differ significantly in the types of glycans that they recognize with high affinity. These proteins function as adhesion and signaling receptors in many immune functions such as inflammation and immunity to tumor and virally infected cells.

HISTORICAL BACKGROUND AND DISCOVERY OF C-TYPE LECTINS

The first C-type lectin identified in animals was the hepatic asialoglycoprotein receptor (ASGPR), also termed the hepatic galactose/N-acetylglucosamine receptor, as introduced in Chapter 26. It was discovered during studies on the structure and function of the sialylated serum glycoprotein, ceruloplasmin. Most serum glycoproteins contain terminal sialic acid residues, and it was observed that desialylated glycoproteins were rapidly removed from the blood. A key experimental result was that the clearance of enzymatically desialylated and radiolabeled ceruloplasmin depended on exposure of the penultimate galactose residue in ceruloplasmin, and removal or modification of the galactose caused retention in the circulation. The desialylated glycoproteins removed from the circulation were sequestered in the liver, principally in lysosomes.

A Ca\(^{2+}\)-dependent receptor specific for asialoglycoproteins was identified in hepatocyte plasma membrane fractions, and the ASGPR was purified by affinity chromatography on asialo-oroosomucoid–Sepharose. The ASGPR was found to consist of a major subunit with a molecular mass of approximately 48 kD and a minor subunit with a mass of about 40 kD. The purified rabbit hepatocyte receptor agglutinated desialylated human and rabbit erythrocytes and also induced mitogenesis in desialylated peripheral lymphocytes. This was the first demonstration that an animal lectin could have such profound effects on cellular metabolism. Binding of rabbit hepatic lectin to cells was inhibited by both N-acetylgalactosamine and galactose, with the former being more potent than the latter, and the lectin bound best to glycoproteins containing either nonreducing terminal N-acetylgalactosamine or galactose. At the time of the discovery, it was not known that circulating glycoproteins had terminal N-acetylgalactosamine residues, but now we know that many pituitary glycoprotein hormones and parasite-derived glycoproteins contain terminal β-linked N-acetylgalactosamine residues and may be recognized by this hepatic receptor. A similar lectin in rats, which contained an unusual heterotrimeric structure composed of two subunits, was termed rat hepatic lectin R2/3. A related lectin identified in chicken hepatocytes recognized glycoproteins containing terminal N-acetylgalactosamine residues. Thus, the avian liver rapidly cleared only glycoproteins that lacked both terminal sialic acid and galactose residues. Interestingly, circulating glycoproteins in birds appear to be constitutively desialylated compared to their mammalian counterparts.

DEFINITION OF C-TYPE LECTINS AND STRUCTURAL MOTIFS

The C-type lectin fold has been found in more than 1000 proteins, and it represents a ligand-binding motif that is not necessarily restricted to binding sugars. The CTLD broadly denotes proteins with a C-type lectin domain, regardless of their ability to bind sugars. In animals, C-type lectins are the major representatives of CTLD-containing proteins. In metazoans, most proteins with a CTLD are not lectins. Proteins use the C-type lectin fold to bind other proteins, lipids, inorganic molecules (e.g., Ca\(_2\)CO\(_3\)), or even ice (e.g., the antifreeze glycoproteins). Glycan binding by the C-type lectins is always Ca\(^{2+}\)-dependent because of specific amino acid residues that coordinate Ca\(^{2+}\) and bind the hydroxyl groups of sugars. The C-type lectin fold is a rigid scaffold that can accommodate a remarkable number of sequence variations. An extreme example is seen in the major tropism determinant (Mtd), which is a receptor-binding protein of Bordetella bacteriophage. This C-type lectin fold may occur in at least 10\(^13\) different sequences, a diversity that rivals the immunoglobulin fold in its conservation of structure using millions of different primary amino acid sequences. Thus, the C-type lectin fold is an evolutionarily ancient structure that is adaptable for many uses.

The C-type lectin fold is unique. It is a compact domain of 110–130 amino acid residues with a double-looped, two-stranded antiparallel β-sheet formed by the amino- and carboxy-terminal residues connected by two α-helices and a three-stranded antiparallel β-sheet (Figure 31.1).
The CRD has two highly conserved disulfide bonds and up to four sites for binding Ca\(^{2+}\), with site occupancy depending on the lectin. Amino acid residues with carbonyl side chains are often coordinated to Ca\(^{2+}\) in the CRD, and these residues directly bind to sugars when Ca\(^{2+}\) is bound in site 2. A ternary complex may be formed between a sugar in a glycan, the Ca\(^{2+}\) ion in site 2, and amino acids within the CRD. Changes in amino acids within the CRD may alter sugar specificity. Key conserved residues that bind sugars include the “EPN” and “WND” motifs within the CRD of C-type lectins (see mouse L-selectin and rat mannose-binding protein C in Figure 31.1). The presence of such motifs in the CRD, along with Ca\(^{2+}\) binding in site 2 and other secondary structures (including hydrogen bond donors and acceptors flanking a conserved Pro residue in the double-loop region), allows predictions as to whether a CRD binds sugar. However, because the binding site is relatively shallow with few contacts to sugars, it is difficult to predict the glycan that binds to a particular C-type lectin. Nevertheless, sequence determinants in the CRD provide clues to the “monosaccharide” specificity of C-type lectins (e.g., mannose vs. galactose). In several C-type lectins, such as P-selectin and the ASGPR, Ca\(^{2+}\) binding induces structural changes in the CRD that stabilize the double loop region. Loss of Ca\(^{2+}\) can lead to destabilization of these loops and loss of ligand binding, even when Ca\(^{2+}\) is not directly involved in complexing the ligand, as seen in the macrophage mannose receptor. This destabilization is also important in pH-induced changes that lead to loss of ligand-binding affinity, because of the pH-induced loss of Ca\(^{2+}\). In CTLD-containing proteins such as human tetranectiont, which is not known to bind sugars, Ca\(^{2+}\) is also important for interactions with several kringle-domain-containing proteins.

C-type lectins exist as both oligomers and monomers. Many C-type lectins occur as trimers, including the trimeric rat mannose-binding protein-A (MBP-A) in complex with α-methyl mannose (Figure 31.2). The CRD of trimeric lectins is angled to the side of the stalk domain through which the protein associates to form the trimer. The CRDs are at the top of the trimer and can function to enhance multivalent interactions with carbohydrate ligands.

**DIFFERENT SUBFAMILIES OF C-TYPE LECTINS**

There are at least 17 groups of proteins with CTLDs, which are distinguished by their domain architecture. Well over 100 different proteins encoded in the human genome contain the CTLD (Figure 31.3). Most of these groups have a single CTLD, but the macrophage mannose receptor (group VI) has eight of these domains. Groups VII (REG), IX (tetranectiont), XI (attractin), XIII (DGCR2; DiGeorge syndrome critical region gene 2), XV (BIMLEC), and XVII (CDBC) have no known glycan ligands. From a functional perspective, we know most about collectins, endocytic receptors, myeloid lectins, and selectins, and these groups are discussed in detail below.
CTLD-containing proteins are found in all metazoans and many nonmetazoans. This latter group includes bacterial toxins (e.g., pertussis toxin), outer-membrane adhesion proteins (e.g., invasin from *Vesinula pseudotuberculosis*), and viral proteins (e.g., envelope protein in Epstein–Barr virus). Interestingly, the viral proteins have more similarity to mammalian CTLD-containing proteins than the bacterial proteins.

There are at least 135 proteins with CTLDs within the genome of *Caenorhabditis elegans*, which has approximately 19,000 protein-coding sequences. Within this group, 183 C-type CTLDs have been found, because some proteins have multiple CTLDs. Interestingly, in response to pathogens, *C. elegans* expresses at least 10 genes that encode proteins with a CTLD out of the 68 pathogen up-regulated genes described to date.

**THE COLLECTINS**

The collectins are C-type lectins that contain a collagen-like domain and usually assemble in large oligomeric complexes containing 9–27 subunits (Figure 31.3). To date, nine different collectins have been identified: mannos-binding protein (MBP), conglutinin, surfactant proteins SP-A and SP-D, and collectins CL-43, CL-46, CL-P1, CL-L1, and CL-K1. The collectins MBP, conglutinin, CL-43, CL-46, CL-K1, SP-A, and SP-D are soluble, whereas CL-L1 and CL-P1 are membrane proteins. Some collectins, such as MBP and SP-A, organize into a “bouquet,” and others, such as bovine conglutinin and SP-D, organize into a “cruciform” shape. One of the best-studied serum collectins is MBP. Bovine CL-43 is structurally one of the simplest collectins, consisting of only three polypeptides, each of which contains a terminal CTLD. Rats have two serum MBPs designated A and C, sometimes called mannan-binding proteins. Humans appear to have only a single MBP corresponding to the rat MBP-A.

The collectins contribute to “inmate” immunity and act before the induction of an antibody-mediated response. Collectins stimulate in vitro phagocytosis by recognizing surface glycans on pathogens, they promote chemotaxis, and they stimulate the production of cytokines and reactive oxygen species by immune cells. Lung surfactant lipids have the ability to suppress a number of immune cell functions such as proliferation, and this suppression of the immune response is further augmented by SP-A. Although SP-A and SP-D were originally found in the lung, they are also expressed in the intestine.

MBP forms a trimeric helical structure via interactions of the collagenous tails that are stabilized by disulfide bonds in the cysteine-rich aminoterminal region. These trimeres aggregate to generate three or six trimers in a “bouquet” organization. Each CRD in the trimer is separated by approximately 53 Å, which is critical to the function of the lectin. This is because each individual CRD has a relatively low affinity and low specificity for glycan ligands and can bind to glycans rich in N-acetylgalacoseamine, N-acetylmannosamine, fucose, and glucose. The spacing between CRDs provides regulation and enhances the potential interactions with extended mannan-containing glycoconjugates, especially those on bacteria, yeast, and parasites.

Binding of MBP and other collectins to a target cell directly activates complement via the classical pathway and generates opsonic C3b fragments that coat pathogens and lead to their phagocytosis. For human MBP, this activation results from a novel type of C1s-like serine protease that complexes with MBP and initiates the complement cascade in vivo. Some individuals with MBP deficiency syndrome have mutations in the Gly-X-Y repeat encoded within exon-1 of the MBP gene (*MBL2*). Mutations within exon-1, which are highly variable among human populations, inhibit assembly of the MBP subunit, leading to increased risk of microbial infections. Furthermore, several polymorphisms within the promoter region of MBL2 are associated with MBP deficiency and enhanced susceptibility to infections.

**THE ENDOCYTIC RECEPTORS**

Many C-type lectins function in receptor-mediated endocytosis to deliver bound, soluble ligands to lysosomes (Figure 31.4). The ASGPR and most endocytic receptors are type II transmembrane proteins, whereas the macrophage mannose receptor is type 1. The cytoplasmic domains of endocytic C-type lectins, such as the ASGPR in hepatocytes, DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin) in myeloid cells, and P-selectin in platelets and endothelial cells, have internalization motifs that include triads of acidic amino acid and dileucine- or tyrosine-based motifs. Myeloid cells, including dendritic cells and macrophages, express a large number of C-type lectins. Endocytosis of ligands by C-type lectins in dendritic cells and macrophages can lead to receptor accumulation and degradation in phagolysosomes or to recycling of the receptor to the cell surface. The pathway taken is dependent on the bound ligand. For example, dectin-1 is degraded when it internalizes zymosan, but it is recycled when it endocytoses soluble ligands. Stimulation of C-type lectins such as dectin-1 in myeloid cells activates mitogen-activated protein kinase (MAPK) and NF-κB and enhances transcription of genes important in innate immune responses. Internalization of antigens via the C-type lectins in dendritic cells induces production of reactive
oxygen species and other responses.

The mammalian ASGPR is composed of major and minor subunits in different ratios that are encoded by two genes. The human ASGPR is a heterotetramer (H1 and H2 in a 3:1 ratio). The H2 transcript, however, exhibits splice variants H2a, H2b, and H2c, and the encoded proteins may contribute to different receptor structures and activities. The rat ASGPR is composed of three subunits, designated rat hepatic lectins (RHL) 1, 2, and 3. RHL1 is derived from a single gene, whereas RHL2 and RHL3 are derived from the same gene and have the same primary sequence, but they differ in glycosylation. In contrast, the chicken hepatic lectin is probably a homotrimer and contains a single subunit. These hepatic lectins occur in oligomeric forms that promote high affinity for specific glycoconjugate ligands. Clustering of the CRDs may determine both the specificity and affinity of the lectins for ligands because each individual CRD can act independently to bind sugar. Studies have shown that the precise orientation of the trimers within the ASGPR can dictate its affinity to specific glycans that have multivalent presentations. Tri- and tetra-antennary N-glycans with appropriate branching and presentation of nonreducing terminal galactose or N-acetylglucosamine residues bind to the rat ASGPR with greater than 100,000 times higher affinity (nm range) than ligands with a single terminal Gal/GalNAc residue. Interestingly, the rat hepatic ASGPR can also bind sialylated ligands (Siaα2-6Galβ1-4GlcNAc-R). The hepatic lectins have a single C-type CRD, whereas the macrophage mannose receptor has eight CRDs in a single polypeptide. The adjacent CRDs may help it to bind specific multivalent, mannose-containing glycans. The macrophage mannose receptor internalizes lysosomal enzymes containing high-mannose-type N-glycans and facilitates phagocytosis of several pathogens such as yeast, *Pneumocystis carinii*, and *Leishmania*.

Although the ASGPR was predicted to contribute to homeostasis and maintenance of serum glycoprotein levels, mice lacking subunit 2 have no obvious phenotypic abnormalities and do not accumulate endogenous glycoproteins in serum. However, the mutant mice have defective hepatic uptake and clearance of injected asialoglycoproteins. Thus, ASGPR may be required to regulate serum glycoprotein levels in periods of induced stress, which is known to elevate serum glycoprotein levels, and/or to be involved in specific interactions with glycoconjugates from pathogenic organisms. In the latter case, these hepatic receptors may function as part of the innate immune system, perhaps in a manner like the C-type lectins in myeloid cell function. For example, the human ASGPR promotes endocytosis-dependent entry of hepatitis B virus particles into liver cells. It is interesting that autoimmune hepatitis is associated with autoantibodies to the human hepatic ASGPR, and there is evidence that hepatitis virus infection can augment this response.

Domains of C-type lectins other than the canonical CRD may also have receptor activity. For example, the cysteine-rich domain of the “mannose” receptor binds to glycans containing R-GalNAc-4-SO₄ on pituitary glycoprotein hormones and thus acts to clear these hormones from the circulation.

THE MYELOID C-TYPE LECTINS

Myeloid cells have many C-type lectins, which belong mainly to groups II, V, and VI (see Figure 31.3). In addition, myeloid cells express many members of the galectin and Siglec families of lectins. C-type lectins in myeloid cells include in group II, DC-SIGN (in humans, but there is no murine homolog), SIGN-R1 (in mice, but there is no human homolog), macrophage C-type lectin (MCL), dectin-2, langerin, and the macrophage galactose-binding lectin (MGL); in group V, dectin-1, myeloid-DAP12-associating lectin (MDL-1), and dendritic-cell-associated lectin-1 (DCAL-1); and in group VI, macrophage mannose receptor and DEC-205.

Dendritic cells (DCs) are important antigen-presenting cells. They internalize antigens by fluid-phase pinocytosis and via specific endocytic receptors, and they process antigens for presentation to CD8⁺ cytotoxic T cells. DCs also contribute to the balance between tolerance and the induction of immunity and help to distinguish harmless “self-antigens” from pathogens (Figure 31.5). When DCs are activated, they migrate to lymph nodes where they interact with T cells. The types of antigens that DCs encounter lead to their differentiation into different subtypes, which function to instruct the differentiation of T cells. Toll-like receptors (TLRs) and C-type lectins (for myeloid cells, these have been termed C-type lectin receptors or CLRs) act to help DCs discriminate between pathogens and self-antigens. TLRs are pattern-
recognition receptors (PRRs) that interact with “pathogen-associated molecular patterns” (PAMPs). TLRs, unlike CLRs, cannot directly promote phagocytosis of bound ligands. Ligation of TLRs can lead to DC maturation in a pathogen-specific manner. In contrast, DC interaction with pathogens through CLRs, in the absence of TLR activation, leads to internalization and processing of antigens and can result in DCs remaining immature. Interactions of T cells with antigens presented by immature DCs may lead to tolerogenic, rather than pathogenic, responses. However, if both TLRs and CLRs are activated and “cross-talk” ensues, DCs mature in different ways. For example, mycobacteria interact with DCs via TLR-2 and TLR-4, resulting in strong T-helper 1 (Th1) responses by the activated DCs. However, some virulent strains of mycobacteria secrete glycosylated factors (e.g., ManLAM) that are bound by CLRs, but not by TLRs, which lead to down-regulation of TLR activation and limitation of DC maturation.

Dectin-1 is a type of natural killer (NK) cell C-type lectin (group V; see Figure 31.3) that binds ligands independently of Ca²⁺. Dectin-1, which is also expressed on human neutrophils and macrophages, is a major PRR that recognizes glycan antigens such as β-glucans. When dectin-1 and TLR-2 are coligated by PAMPs, they coordinate the secretion of proinflammatory cytokines (interleukin-12 and tumor necrosis factor-α [TNF-α]) and the production of reactive oxygen species. Dectin-1 is degraded intracellularly upon internalization of large-sized β-glucans, but it can recycle upon internalizing small-sized β-glucans. Dectin-1 carries an immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic domain, which is involved in cell signaling through Syk family tyrosine kinases. DC-SIGN in human cells has been of special interest because it binds to the oligomannose-type N-glycans in the envelope glycoprotein of HIV-1. This protein also recognizes fucosylated ligands expressed by parasites such as the helminth Schistosoma mansoni.

Langerhans cells are a special type of DC and represent immature DCs found in the epidermis and mucosal tissues. These cells probably do not commonly coexpress the macrophage mannose receptor, but they do express many other C-type lectins, including langerin, which is a C-type lectin that largely recognizes glycans rich in mannose. Internalization of ligands by langerin leads to accumulation in Birbeck granules, which are subdomains of endosomes specifically found in Langerhans cells. Interestingly, polymorphisms in the gene encoding langerin are associated with changes in ligand specificity and loss of Birbeck granules.

**THE SELECTINS**

The selectins are perhaps the best-characterized family of C-type lectins because of their extensively documented roles as cell-adhesion molecules that mediate the earliest stages of leukocyte trafficking. The selectins are type-1 membrane proteins that are expressed on endothelial cells, leukocytes, and platelets. Interactions between the selectins and cell-surface glycoconjugate ligands play key roles in adhesive interactions among these cells (Figure 31.6). These interactions promote tethering and rolling of leukocytes and platelets on vascular surfaces, and are important for lymphocyte homing to secondary lymphoid organs and for leukocyte recruitment to sites of inflammation and injury. Rolling is a form of adhesion that requires rapid formation and dissociation of bonds between selectins and their ligands. Rolling adhesion enables leukocytes to encounter endothelium-bound chemokines. Signaling through chemokine receptors cooperates with signaling through selectin ligands to activate leukocyte integrins, which bind to immunoglobulin superfamily ligands on endothelial cells to slow rolling velocities and arrest leukocytes on vascular surfaces. The arrested leukocytes then emigrate across the vascular wall into the underlying tissues.

The three selectins are L-selectin, which is expressed on all leukocytes; E-selectin, which is expressed by cytokine-activated endothelial
cells; and P-selectin, which is expressed constitutively in α-granules of platelets, in Weibel–Palade bodies of endothelial cells, and on the surfaces of activated platelets and endothelial cells (Figure 31.7). Each selectin has a C-type CRD at the amino terminus followed by a consensus epidermal growth factor (EGF)-like domain and a number of short consensus repeats composed of sushi domains (also called complement control protein [CCP] modules). The proteins have a single transmembrane domain (TM) and a cytoplasmic domain and are relatively rigid, extended molecules. The C-type CRD of each selectin has modest affinity for the sialylated, fucosylated structure known as the sialyl Lewisα antigen NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-R (SLεα). In addition, P- and L-selectin, but not E-selectin, bind to some forms of heparin/heparan sulfate. However, each of the selectins binds with higher affinity to specific macromolecular ligands. Many of the known ligands are mucins containing sialylated, fucosylated O-glycans. The major ligand for P-selectin, termed P-selectin glycoprotein ligand-1 (PSGL-1), has sulfated tyrosine residues adjacent to a core-2-based O-glycan expressing SLεα. Ligands for L-selectin that occur within specialized endothelia termed high endothelial venules (HEV) contain 6-sulfo-SLeα antigens on mucin-type O-glycans and on N-glycans.

**FIGURE 31.7**
Overall domain structures of P-selectin, E-selectin, L-selectin, and PSGL-1. (a) The domain organization of the selectins and their expression patterns are indicated. P-Selectin also forms homodimers in the membrane. The predicted disulfide-bonded dimeric (more...)

**P-Selectin**

P-Selectin (CD62P) was discovered as an antigen expressed on the surface of activated platelets. It is constitutively expressed in megakaryocytes, where it is packaged into the membranes of α-granules of circulating platelets. It is also expressed in the Weibel–Palade bodies of vascular endothelial cells. Within minutes following activation of either platelets or endothelial cells by proinflammatory secretagogues such as histamine, thrombin, or complement components, P-selectin is expressed on the cell surface because of fusion of the intracellular storage membranes with the plasma membrane. Sequences within the cytoplasmic domain of P-selectin mediate its sorting to secretory granules as well as its rapid endocytosis from the plasma membrane and movement from endosomes to lysosomes, where it is degraded. Splice variants of human P-selectin transcripts yield forms of P-selectin that lack a transmembrane domain, thus contributing to low-level soluble forms of P-selectin in the circulation. Leukocyte adhesion also stimulates proteolytic cleavage of the ectodomain of P-selectin from the plasma membrane, releasing it into the circulation. The inflammatory mediators TNF-α, interleukin 1-β (IL1-β), and lipopolysaccharide (LPS) augment transcription of mRNA for P-selectin in endothelial cells in mice but not in humans.

P-Selectin contributes to leukocyte recruitment in both acute and chronic inflammation. Mice that lack P-selectin exhibit defective rolling on endothelial cells of postcapillary venules, diminished recruitment of neutrophils or monocytes into tissues following injection of inflammatory mediators, and impaired recruitment of T cells into skin or other tissues following challenge with specific antigens. Mobilization of P-selectin to the surfaces of activated endothelial cells is important for all these responses. In addition, P-selectin expressed on the surfaces of activated platelets contributes to inflammation as well as to hemostasis and thrombosis. Activated platelets adhere through P-selectin to neutrophils, monocytes, NK cells, and some subsets of T lymphocytes. This adhesion augments the recruitment of leukocytes and platelets to sites of vascular injury. Expression of P-selectin on both platelets and endothelial cells contributes to experimental atherosclerosis in mice. Platelet-expressed P-selectin also stimulates monocytes to synthesize tissue factor, a key cofactor of blood coagulation that facilitates fibrin deposition during clot formation.

As discussed below, the major leukocyte counterreceptor for P-selectin is PSGL-1. P-Selectin binds weakly to some forms of heparin/heparan sulfate and to some glycoproteins that bear the SLeα determinant. The physiological significance of this binding is unclear, but the levels of heparin that are clinically prescribed might be able to block P-selectin functions. In addition, P-selectin can interact with mucins containing highly clustered glycans bearing SLeα antigens, which might be important in metastasis of tumors bearing such ligands (see Chapters 43 and 44). Heparin might be therapeutically useful in blocking cancer metastases through blocking adhesion of tumor cells to P-selectin.

**PSGL-1**
PSGL-1 (CD162) is a homodimeric, disulfide-bonded mucin with subunits with a molecular mass of approximately 120 kD (351 amino acids). It is the major physiological ligand on leukocytes for P- and L-selectin and is also an important ligand for E-selectin (Figure 31.7). The precursor to the PSGL-1 monomer has 402 amino acids, including an 18-­amino-acid signal sequence. Maturation of the protein occurs following cleavage at residues 38–41 by a paired basic amino-acid-converting enzyme in leukocytes. There are 16 decapetide repeating units with the consensus sequence spanning residues 118-­277 in the ectodomain of the long form of the protein, which is the major form expressed in humans. Murine PSGL-1 has 397 amino acids with recognizable sequence similarity to the human sequence, but the mouse protein has only 10 decameric repeats with the consensus sequence -E-T-S-Q/K-P-A-P-T/M-E-A-, which is different from human PSGL-1. The highest homology between human and murine PSGL-1 occurs in the transmembrane and cytoplasmic domains.

PSGL-1 is primarily expressed in hematopoietic cells (including some hematopoietic stem cells), all neutrophils, monocytes, eosinophils, and basophils and certain subsets of T cells. PSGL-1 is also expressed in some activated endothelial cells, most notably the inflamed microvessels of the ileum in a spontaneous model of chronic ileitis in mice. When it undergoes the appropriate posttranslational modifications (see below), PSGL-1 interacts with each of the three selectins to support leukocyte rolling under flow. Engagement of PSGL-1 during rolling also transduces signals into leukocytes that activate leukocyte integrins to slow rolling velocities. Signaling through PSGL-1 also cooperates with signaling through chemokine receptors to elicit other effector responses in leukocytes.

PSGL-1 is heavily glycosylated; each subunit of human PSGL-1 has three potential sites for N-glycosylation and 70 serine and threonine residues in the extracellular domain that are potential sites for O-glycosylation. In addition, human PSGL-1 has three amino-terminal Tyr residues and murine PSGL-1 has two tyrosine residues that are potentially sulfated. Like most mucins, PSGL-1 has an extended structure (Figure 31.7).

Most of the O-glycans of native PSGL-1 purified from human HL60 cells are core-2 structures, of which only a subset are fucosylated. Optimal binding of P-selectin to PSGL-1 requires that the latter presents SLe^a on a specific, amino-terminal core-2 O-glycan and sulfate esters on specific amino-terminal tyrosine residues. Tyrosine sulfate residues are generated by two tyrosylprotein sulfotransferases (TPST-1 and -2), which transfer sulfate from the sulfate donor (phosphoadenosine phosphosulfate) to exposed tyrosine residues. Thus, the key binding domain of PSGL-1 resides in its amino-terminal region. There are many lines of evidence that support this conclusion. Antibodies to this region block binding of PSGL-1 to P- and L-selectin (but not to E-selectin, which interacts with more than one site on PSGL-1 and does not interact with sulfated tyrosines). Treatment of neutrophils with a cobra venom metalloproteinase that removes the first ten amino acids from the amino terminus of PSGL-1 abrogates its binding to P- and L-selectin. Site-directed mutagenesis of a specific O-glycosylated amino-terminal threonine and/or the three tyrosine residues in the amino terminus of PSGL-1 prevents binding to P- and L-selectin. Finally, synthetic glycosulfopetides with the structure shown in Figure 31.8 bind to P-selectin with the same high affinity (~300 nM) as that of native PSGL-1.

**FIGURE 31.8**
The molecular interactions between P-selectin and the amino terminus of PSGL-1. The C-type carbohydrate-recognition domain (CRD) at the amino terminus of P-selectin is a relatively large binding site that interacts with tyrosine sulfate residues, peptide (more...)

The data point to a model in which the combination of tyrosine sulfate residues and oligosaccharides on the protein are required for high-affinity binding to P-selectin (Figure 31.7). The cocrystal structure of a PSGL-1-derived glycosulfopetide with P-selectin confirms the complex and tight association between these binding partners. The interactions between the glycosulfopetide and P-selectin result from a combination of hydrophobic and electrostatic contacts. These include contacts of at least two of the three tyrosine sulfate residues as well as other PSGL-1 amino acids with multiple residues within the P-selectin CRD. In addition, two hydroxyl groups in the fucose residue of SLe^a ligate the lectin domain-bound Ca^{2+}, and there are additional binding interactions with the hydroxyl groups of galactose and the –COOH group of Neu5Ac.

**E-Selectin**

E-Selectin (CD62E) was discovered as a leukocyte adhesion molecule expressed by activated vascular endothelial cells. In most tissues (the bone marrow and skin may be exceptions), endothelial cells do not constitutively express E-selectin. Cytokine-dependent transcriptional processes lead to an inducible expression of E-selectin on the surface of the endothelium. Inducible transcription of the E-selectin locus by
TNF-α, IL1-β, and LPS is mediated at least in part through NF-κB-dependent events. In vitro, cytokine-dependent regulation of the E-selectin locus yields E-selectin expression beginning about 2 hours after cytokine treatment, with maximal expression at about 4 hours. E-Selectin expression then declines to basal levels within 12–24 hours in vitro, but E-selectin may be expressed chronically at sites of inflammation in vivo. Decline of E-selectin expression is associated with decreased transcription of the E-selectin locus, degradation of E-selectin transcripts, and internalization and turnover of E-selectin protein. Acute and chronic inflammatory conditions associated with E-selectin expression include sepsis, rheumatoid arthritis, and organ transplantation. E-Selectin cooperates with P- and L-selectin to recruit leukocytes to sites of inflammation.

Physiological ligands for E-selectin contain the SLLeα antigen and occur on neutrophils, monocytes, eosinophils, memory/effector T cells, and NK cells. Each of these cell types is found in acute and chronic inflammatory sites in association with the expression of E-selectin, which implicates E-selectin in the recruitment of these cells to such inflammatory sites. PSGL-1 is one of the physiological ligands for E-selectin, but E-selectin can also interact with several other glycoproteins that express the SLLeα antigen on either N- or O-glycans, including the E-selectin ligand-1, CD44, L-selectin (in humans), and possibly long-chain glycosphingolipids expressing the SLLeα antigen. These interactions may have a potential role in cancer metastasis (see Chapter 44).

### L-Selectin

L-Selectin (CD62L) is expressed on the microvilli of most leukocytes, including all myeloid cells, naïve T and B cells, and some memory/effector T cells. L-Selectin was discovered through efforts to define molecules that facilitate recirculation of lymphoid cells from the intravascular compartment to the secondary lymphoid organs, including lymph nodes and Peyer’s patches, from which the lymphoid cells then return to the circulation through the lymphatic system. This recirculation process provides lymphocytes with the opportunity to encounter foreign antigens displayed by antigen-presenting cells within secondary lymphoid organs. Early studies indicated that blood lymphocytes enter lymph nodes in postcapillary venules within these organs. These postcapillary venules are lined with a specialized endothelium (HEV). Cells in the HEV are cuboid in shape and their surfaces are decorated with certain glycoproteins required for adhesion of lymphocytes to the HEV, leading to subsequent transmigration. L-Selectin also cooperates with P- and E-selectins to promote leukocyte recruitment of myeloid cells and memory/effector T cells to sites of infection or injury during acute or chronic inflammation. L-Selectin is proteolytically shed from the surface of activated leukocytes by a metalloproteinase TNF-α-converting enzyme (TACE).

Rolling mediated by L-selectin exhibits a counterintuitive “shear threshold” requirement (e.g., a minimum flow rate is required for leukocytes to roll). As the flow rate drops below this threshold, leukocytes roll faster and more unstably and then detach. Flow-enhanced rolling operates through a force-dependent mechanism. As the flow rate increases, the force applied to adhesive bonds between L-selectin and its ligands increases. At threshold levels, the force actually strengthens the bonds, which prolongs their lifetimes. These are called “catch bonds.” As the flow rate increases further, the applied force begins to weaken the bonds, which shortens their lifetimes. These are called “slip bonds.” Catch bonds are regulated by force-dependent straightening of the angle between the lectin and EGF domains of L-selectin, which affects how ligand dissociates from the binding interface on the lectin domain. Transitions between catch and slip bonds are also seen for interactions between P-selectin and PSGL-1 and probably also occur between E-selectin and its ligands. However, the E-selectin transitions occur at lower forces with less dramatic effects at physiologically relevant shear forces in the circulation.

Lymphocytes home to secondary lymphoid organs where they may encounter antigens. Lymphocytes can return to the blood through the thoracic duct. This process is termed lymphocyte recirculation or homing. Entry to the lymph occurs in these organs through the HEV, where cuboid cells express ligands for lymphocyte L-selectin. These ligands are called peripheral node addressins, a group of mucins expressed on the HEV of lymph nodes. They include the mucins CD34, Sgp200, GlyCAM-1, MAHDAM-1, endoglycan, endomucin, and the podocalyxin-like protein (PCLP).

A unique feature of the L-selectin ligands on HEV is the requirement for sulfated glycans, such as 6-sulfo-SLeα on both core-2 O-glycans and on extended core-1 O-glycans. The 6-sulfo-SLeα determinant is associated with the MECA-79 epitope on O-glycans (Figure 31.9), an antibody that binds to 6-sulfo-N-acetyllactosamine on extended core-1 O-glycans. The biosynthesis of the 6-sulfo-SLeα determinant depends on two key α1-3 fucosyltransferases, FucT-VII and FucT-IV, along with at least four different sulfotransferases that may form the 6-sulfo-SLeα determinant. Two of these sulfotransferases, GlcNAc6ST-1 and GlcNAc6ST-2, are both expressed in HEV and appear to be most important. Mice lacking FucT-VII or both FucT-VII and FucT-IV have dramatically reduced homing of lymphocytes to lymph nodes. Mice lacking both GlcNAc6ST-1 and GlcNAc6ST-2 do not express 6-sulfo-SLeα or the MECA-79 epitope and exhibit markedly diminished lymphocyte homing to lymph nodes. A unique β1-3GlcNAcT generates the extended core-1 O-glycan; mice lacking both this β1-3GlcNAcT and the β1-6GlcNAcT branching enzyme for core-2 O-glycan biosynthesis do not express the MECA-79 antigen, but they have residual
lymphocyte rolling on HEV and only a minimal decrease in lymphocyte numbers in peripheral and mesenteric lymph nodes. The residual L-selectin-dependent lymphocyte homing appears to result from 6-sulfo-SLeα on N-glycans, suggesting that both N- and O-glycans on HEV glycoproteins contribute to L-selectin-dependent lymphocyte recirculation through lymph nodes.

L-Selectin also plays a role in adhesion of neutrophils, eosinophils, and monocytes to nonlymphoid vascular endothelium (see Figure 31.6). The major ligand for L-selectin in these inflammatory settings is PSGL-1 (see Figure 31.7), which is expressed on adherent leukocytes and may also be deposited on inflamed endothelial cells as fragments left behind by previously rolling leukocytes. Unlike the HEV ligands, PSGL-1 interacts with L-selectin through cooperative binding of its amino-terminal core-2 O-glycan capped with SLeα and its amino-terminal sulfated tyrosines, analogous to but not identical to its interactions with P-selectin. In addition, an alternate ligand on endothelial cells is heparan sulfate. Mice lacking L-selectin have defects in neutrophil recruitment in the context of inflammation as well as defects in homing of naïve lymphocytes to secondary lymphoid organs.

THE NATURAL KILLER LYMPHOCYTE PROTEINS WITH C-TYPE LECTIN DOMAINS

The immunoglobulin-like receptors and NK cell receptors containing a CTLD are two families of major histocompatibility complex (MHC) class-I-specific receptors found on NK cells. NK receptors with the CTLD are largely in group V and are represented by the Ly49 family of receptors in mice and the NK complex (NKC) in humans (see Figure 31.3). The human NKC proteins include CD69, CD94, activation-induced C-type lectin (AICL), and mast-cell-function-associated antigen (MAFA). The murine Ly49 family includes the Ly49 group of about a dozen proteins, including CD94, NKG2-A, -C, -D, and -E.

NK receptors with a CTLD are either activating or inhibitory to immune responses. For the most part, these proteins function in targeting lymphocytes to cells that lack MHC class I antigens. The Ly49 and NKC proteins are disulfide-bonded, type II, homodimeric transmembrane proteins. Most of these proteins express immunoreceptor tyrosine-based inhibitory motif (ITIM) motifs in their cytoplasmic domain. Dectin-1, which is not considered to be part of the Ly49 or NKC complex, is exceptional in that it contains an activating ITAM-like motif. In human NK cells, CD94 forms MHC class-I-specific, disulfide-linked, heterodimers with NKG2 family members, which also contain a CTLD. Ligand binding to the CTLDs of these NK receptors can trigger or inhibit target cell lysis by NK cells or can activate various hematopoietic cells.

Most of the NK receptors with CTLDs are not known to bind carbohydrate ligands and lack the conserved Ca2+-binding residues and amino residues in most C-type lectins that bind glycans. In humans, it is known that Ca2+ is not required for CD94/NKG2 interactions with HLA-E. The CTLD in the NK receptor has a somewhat different structure from typical C-type lectins and may have evolved to promote interactions with MHC class I glycoproteins. However, some recent studies suggest that murine CD69 may bind some sulfated glycans; in addition, another protein in the NK family, the osteoclast inhibitory lectin (OCIL) from humans and mice, also binds some sulfated polysaccharides in a Ca2+-independent fashion. Thus, the possibility that the NK receptors with CTLDs bind to some types of glycan ligands should be explored. As discussed above, dectin-1 lacks the canonical residues for Ca2+ binding and monosaccharide binding but nevertheless binds β-glucans. Thus, the CTLDs in the NK receptors may have evolved to recognize glycans by novel mechanisms.

The B-cell differentiation antigen (CD72) interacts with the B-cell receptor (BCR) and modulates BCR-mediated signals. CD72 is a type II membrane proteins with a CTLD that lacks the residues associated with Ca2+ and monosaccharide binding. It has been speculated that CD72 might recognize BCR or other glycoprotein ligands by corecognizing glycan and protein determinants. The low-affinity IgE receptor, CD23, also contains a CTLD that interacts with CD21 and other ligands, but it may bind these ligands in a manner that depends on their correct glycosylation, perhaps a combination of glycan and peptide determinants.

PROTEOGLYCANs WITH C-TYPE LECTIN DOMAINS

The CTLD has also been identified in several proteoglycans (or lecticans) that lack trans-membrane domains and occur in the extracellular matrix (group I; see Figure 31.3). These include aggregan, brevican, versican, and neurocan. Like the selectins, each of these core proteins contains a CTLD, an EGF-like domain, and a CCP domain, but their order is different and they are located in the carboxyl terminus of the
protein. A large region containing attachment sites for chondroitin sulfate and keratan sulfate is proximal to the lectin domain. A more complete discussion of the proteoglycans is provided in Chapter 16. The exact functions of the CTLD in these proteins are still unknown. The CTLD of rat aggrecan is important for Ca\(^{2+}\)-dependent binding to the fibronectin type II repeats 3–5 of rat tenascin-R. The CTLDs in other lectins are probably also responsible for protein–protein interactions with other receptors, including tenascin-R, tenascin-C, and other tenascins, which are extracellular matrix glycoproteins highly expressed in the nervous system. Interestingly, the protein–protein interactions between rat fibronectin and the CTLD of rat aggregan bear resemblance to the protein–protein interactions seen in P-selectin binding to PSGL-1. Tenasin-R is one of the main carriers of the unusual glycan antigen HNK-1, which was named after its identification on human NK cells. Recent studies show that brevican, a lecitan found in the nervous system, binds to the HNK-1-containing glycosphingolipids. Thus, the CTLD in lectins may represent a versatile structural feature that can be used for both protein–protein and protein–glycan interactions.

OTHER TYPES OF C-TYPE LECTINS

A number of proteins with C-type CRDs have been identified in the pancreas and kidney, but the importance of the CRD and glycans binding to the function of these proteins is unclear. Autosomal dominant polycystic kidney disease (ADPKD) is a commonly occurring hereditary disease that accounts for about 10% of end-stage renal disease. PKD1, one of two recently isolated ADPKD gene products, has been implicated in cell–cell and cell–matrix interactions. The PKD1 gene encodes a novel protein named polycystin that has multiple cell-recognition domains, including a single CTLD at its amino terminus. The function of PKD1 and the effect of mutations on its possible activity are unclear. Interestingly, some C-type lectins can be extremely small; for example, HIP (hepatic intestinal pancreatic protein) and PSP (pancreatic stone protein), which are essentially isolated CTLDs that are preceded by a signal sequence.

Lower vertebrates, invertebrates, and some viruses have now been found to contain the CTLD, and some of these proteins have been shown to bind sugars. For example, the galactose-specific lectin from Crotaulus atrox binds a variety of galactose-containing glycolipids in a Ca\(^{2+}\)-dependent fashion. A number of related venom proteins inhibit platelet function and/or the coagulation cascade. Alboaggregin A from Trimeresurus albolabris (the white-lipped pit viper) contains four subunits, and subunit 1 contains a single CTLD at its amino terminus. The protein binds to the platelet GP Ib/IX receptor and stimulates platelet agglutination, but the potential role of glycan recognition in this process is presently unknown.

FURTHER READING

14. Ludwig IS, Geijtenbeek TBH, van Kooyk Y. Two way communication between neutrophils and dendritic cells. Curr Opin


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Chapter 32

I-type Lectins

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Chapter 32  I-type Lectins

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I-type lectins are glycan-binding proteins that belong to the immunoglobulin superfamily (IgSF), excluding antibodies and T-cell receptors. Bioinformatics analyses of mammalian genomes predict more than 500 proteins of the IgSF, other than antibodies and T-cell receptors. Thus, there is considerable potential for assignment to the I-type lectin family. In fact, the Siglec family of sialic acid–binding lectins is the only well-characterized group of I-type lectins, both structurally and functionally. These proteins are thus the major focus of this chapter. Details of their discovery, characterization, binding properties, and biology are provided, along with discussions of their functional implications in mammalian biology.

HISTORICAL BACKGROUND AND OVERVIEW

Members of the IgSF must contain at least one immunoglobulin (Ig)-like fold, but often contain other structural features such as fibronectin type III repeats. The Ig fold was first discovered in antibodies and is made up of antiparallel β-strands organized into a β-sandwich containing 100–120 amino acids and usually stabilized by an intersheet disulfide bond. Three types or “sets” of Ig domains have been defined on the basis of similarities in sequence and structure to the domains of antibodies: the V-set variable-like domain, the C1- and C2-set constant-like domains, and the I-set domain that combines features of both V- and C-set domains.

Prior to the 1990s, it was thought that antibodies were the only IgSF members capable of recognizing glycans. The first direct evidence for non-antibody IgSF glycan-binding proteins was from independent studies on sialoadhesin (Sn), a sialic acid (Sia)-dependent binding receptor on certain mouse macrophage subtypes, and on CD22, a molecule already previously cloned as a B-cell marker. A variety of techniques were used to show that Sn functions as a lectin, including loss of binding following sialidase treatment of ligands, inhibition assays with sialylated compounds, and Sia-dependent binding of the purified receptor to glycoproteins and to red blood cells derivatized to carry Sias in α2-3 linkages. In the case of recombinant CD22, loss of cell adhesive interactions caused by sialidase treatment led to the discovery that it was a Sia-binding lectin, with a high degree of specificity for α2-6-linked Sias. The cloning of Sn then showed that it was an IgSF member that had homology with CD22 and with two other previously cloned proteins, CD33 and myelin-associated glycoprotein (MAG). Demonstration of Sia recognition by CD33 and MAG resulted in the definition of a new family of Sia-binding molecules, which were initially termed the “sialoadhesins.” Meanwhile, evidence for glycan binding by additional IgSF members had emerged, and a suggestion was made to classify all of these molecules as “I-type” lectins. However, it became clear that the Sia-binding molecules were a distinct group sharing both sequence homology and Ig domain organization and that they were not all involved in adhesion. The term Siglec (sialic acid–binding, immunoglobulin-like lectin) was therefore proposed in 1998. Subsequently, most of the CD33-related Siglecs (CD33rSiglecs) were discovered as a direct result of the large-scale genomic sequencing projects, which allowed in silico identification of novel Siglec-related genes and cDNAs.

I-TYPE LECTINS OTHER THAN SIGLECS

Several IgSF members other than Siglecs have been claimed to bind glycans, but in many cases, the evidence is indirect. The best evidence is probably for L1 cell-adhesion molecule (L1-CAM) in the nervous system. However, further studies have indicated that this molecule does not bind Sias through an IgSF domain and it therefore does not strictly qualify as an I-type lectin. The neural cell-adhesion molecule (NCAM) has been claimed to recognize and bind oligomannose-type glycans on adjacent glycoproteins in the nervous system. Similar findings have recently been reported for another IgSF molecule called basigin. The cell-adhesion molecule ICAM-1 has been shown to bind hyaluronan and possibly certain mucin-type glycoproteins. Hemolin is an IgSF plasma protein from lepidopteran insects that binds lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid from Gram-positive...
bacteria. Hemolin appears to have two binding sites for LPS, one that interacts with the phosphate groups of lipid A and another that interacts with the O-specific glycan antigen and the outer-core glycans of LPS. There is indirect and less convincing evidence for interactions of other IgSF molecules with glycans, such as P0 with HNK-1, CD83 with Sias, PILR with Sias on CD99, and CD2 with Lewis^a. Further studies are needed to ascertain whether these are indeed I-type lectins. The rest of this chapter will be devoted to the Siglecs, which are the best-characterized I-type lectins.

**TWO MAJOR SUBFAMILIES OF SIGLECS**

The Siglecs can be divided into two major subgroups based primarily on sequence similarity (Figure 32.1) and on conservation between different mammalian species. The first group comprises Sn (Siglec-1), CD22 (Siglec-2), MAG (Siglec-4), and Siglec-15 for which there are clear-cut orthologs in all mammalian species examined and which share only about 25–30% sequence identity among each other. The second group comprises the CD33rSiglecs, which share about 50–80% sequence similarity but appear to be evolving rapidly and undergoing shuffling of Ig-domain-encoding exons, making it difficult to define orthologs even between rodents and primates (see details below).

**COMMON FEATURES OF SIGLECS**

**The Amino-terminal V-set Sialic Acid–binding Domain**

All Siglecs are type-1 membrane proteins that contain a Sia-binding, amino-terminal V-set domain and varying numbers of C2-set Ig domains that act as spacers, projecting the Sia-binding site away from the plasma membrane. The V-set domain and the adjacent C2-set domain contain a small number of invariant amino acid residues, including an “essential” arginine on the F β-strand that is required for sialic acid binding and an unusual organization of cysteine residues. Instead of the typical intershell disulfide bond between the B and F β-strands, Siglecs display an intrasheet disulfide bond between the B and E β-strands, permitting increased separation between the β-sheets. The resulting exposure of hydrophobic residues allows specific interactions with constituents of Sia. All Siglecs also appear to contain an additional unusual disulfide bond between the V-set domain and the adjacent C2-set domain, which would be expected to promote tight packing at the interface between the first two Ig domains. Although the significance of this for ligand recognition is unclear, it has been noted that the Sia-binding activity of some Siglecs (e.g., CD22 and MAG) appears to require the adjacent C2-set domain, probably for correct folding.

**Absolute Requirement for Sialic Acids in Glycan Ligands**

Siglecs differ from most other mammalian Sia-binding lectins (such as selectins) with respect to their absolute requirement for Sia. Whereas the selectins use Sias as carriers of negative charge to make ionic interactions, the Siglecs make more extensive molecular contacts, exploiting not only the negatively charged carboxylate group, but also the glycerol side chain, the N-acyl group, and the C-4-hydroxyl group. In contrast to selectins, substitution of Sia in oligosaccharides with a sulfate moiety results in loss of binding to Siglecs (although addition of sulfate esters to other parts of the underlying glycan can enhance affinity). In addition, treatment of target cells or glycoconjugates with broad specificity sialidases is an effective way to destroy glycan recognition by Siglecs. Similar to many lectins, the affinity of Siglecs for sialylated ligands is low, with binding constants typically in the high micromolar to low millimolar range, as revealed in surface plasmon resonance, equilibrium dialysis, nuclear magnetic resonance (NMR), or thermal calorimetry measurements (see Chapter 27). Multimerization of Siglecs is likely to occur naturally on plasma membranes, leading to high avidity binding to clustered glycan ligands.

**Masking and Unmasking**

The cell-surface glycoalyx of most mammalian cells is richly decorated in glycoconjugates that contain Sias. The high local concentration of Sias is likely to greatly exceed the $K_d$ value of each Siglec, resulting in “masking” of the Sia-binding site. Consequently, the Sia-dependent binding activity of most naturally expressed Siglecs is difficult to demonstrate unless the cells are first treated with sialidase to eliminate the cis-interacting sialylated glycans. A notable exception is Sn, which was discovered as a Sia-dependent cell-adhesion molecule on macrophages isolated from various tissues. The “masked” state of most Siglecs is a dynamic equilibrium with multiple ligands. Thus, an
external probe or cell surface bearing high-affinity ligands or very high densities of Sia residues can effectively compete even with the binding domains of “masked” Siglects. In addition, changes in expression of glycosyltransferases or sialidases could influence masking and unmasking of Siglects at the cell surface, especially during immune and inflammatory responses.

Expression in a Cell-type-restricted Manner

Siglects show restricted patterns of expression in unique or related cell types. This is most striking for Sn, CD22, and MAG, which are expressed on macrophages, B lymphocytes, and myelin-forming cells, respectively. This theme also extends to some of the CD33rSiglects (most notably in humans), Siglec-6 on placental trophoblasts, Siglec-7 on NK (natural killer) cells, Siglec-8 on eosinophils, and Siglec-11 on tissue macrophages, including brain microglia. In the mouse, Siglec-H and CD33 are excellent markers of plasmacytoid dendritic cells (DCs) and neutrophils, respectively, and Siglec-F is a useful marker of eosinophils. These cell-type-restricted expression patterns are thought to reflect discrete, cell-specific functions mediated by each of these Siglects. However, certain key cells of the immune system such as monocytes and conventional DCs express multiple CD33rSiglects in humans.

Cytoplasmic Tyrosine-based Signaling Motifs

Most Siglects have one or more tyrosine-based signaling motifs. Exceptions are Sn, Siglec-14, Siglec-15, mCD33, and Siglec-H. The most prevalent motif is the immunoreceptor tyrosine-based inhibitory motif (ITIM) with the consensus sequence (V/I/L)XXXY(L/V), where X is any amino acid. More than 100 ITIM-containing membrane receptors have been identified in the human genome, and many of these are established inhibitory receptors of the hematopoietic and immune systems. They function by recruiting certain SH2-domain-containing effectors, the best characterized of which are the protein tyrosine phosphatases SHP-1 and SHP-2 or the inositol 5’ phosphatase SHIP. These counteract activating signals triggered by receptors containing immunoreceptor tyrosine-based activatory motifs (ITAMs). Some Siglects without a prominent cytoplasmic domain instead have a positively charged residue within the transmembrane region, which can associate with the DAP-12 (DNAX activation protein-12) ITAM-containing adaptor.

STRUCTURAL BASIS OF SIGLEC BINDING TO SIALYLATED GLYCANS

The three-dimensional structures of the mouse (m) Sn and human (h) Siglec-7 V-set domains have been determined by X-ray crystallography, in the presence and absence of Sia ligands (Figure 32.2). These provide a structural template for Sia recognition by Siglects that is likely to be shared by other family members. In both instances, the “essential” arginine residue is located in the middle of the F β-strand, making a bidentate salt bridge with the carboxylate of Sia. In the absence of bound ligand, the essential arginine of both Sn and Siglec-7 is masked by a basic residue (either arginine or lysine). On binding ligands, this basic residue moves away to allow access of the essential arginine to the Sia carbohydrate group. Siglects also contain a conserved hydrophobic amino acid (either tryptophan or tyrosine) on the G β-strand that interacts with the glycerol side chain of Sia. The C-4 hydroxyl of Sia makes a hydrogen bond either directly or indirectly via a water molecule, and the N-acetyl group interacts with a tryptophan of Sn via hydrophobic interactions and with a similarly positioned tyrosine of Siglec-7 via hydrogen bonding. Although all Siglects probably share this common template for binding glycosidically linked Sias, their binding preferences for extended glycan chains vary greatly. The peptide loop between the C and C’ β-strands is highly variable among Siglects and has a key role in determining their fine sugar specificity. For example, molecular grafting of the C-C’ loop between Siglects-7 and -9 resulted in switched sugar-binding specificities. Structural studies have shown that this loop appears to be highly flexible, being able to make specific and varied interactions with long glycan chains.

EXPRESSION PATTERNS AND FUNCTIONS OF THE CONSERVED SIGLECS

Sialoadhesin (Siglec-1, CD169)

Sn was identified in 1983 as a Sia-dependent sheep erythrocyte receptor (SER) expressed by mouse stromal macrophages isolated from various tissues. Studies with anti-Sn monoclonal antibodies established that expression in
humans and mice is highly specific for macrophage subsets, especially those in lymphoid tissues and those recruited to inflammatory sites, as seen in inflamed joints in individuals with rheumatoid arthritis and other autoimmune inflammatory disorders. Full-length Sn is predicted to be a transmembrane protein with 17 Ig-like domains. Multiple splice variants encoding truncated and secreted forms of Sn exist, but their biological significance is unknown. Sn can be expressed at very high levels on macrophages, with up to 1 million molecules per cell, generating the potential to mediate cell–cell and cell–matrix interactions. Microscopy studies provided evidence for Sn clustering in vivo, especially in the bone marrow where Sn is localized at the contact sites of resident stromal macrophages and developing granulocytes. Several glycoprotein ligands have been identified from cell lysates using affinity chromatography methods with soluble recombinant forms of Sn. In all cases, the glycoconjugates that bind in a Sia-dependent manner are transmembrane mucins known to display multiple clustered O-linked glycans. These include MUC1 expressed by breast cancer cells and P-selectin glycoprotein ligand-1 (PSGL-1) and CD43 expressed on myeloid cells and T cells. It is unclear if these are indeed specific ligands or if they simply have the highest densities of cognate Sia residues.

The unusually large number of 17 Ig domains in Sn appears to be conserved in mammals and is thought to be important for extending the Sia-binding site away from the plasma membrane to promote intercellular interactions. Electron microscopy shows that Sn is an extended molecule of about 50 nm. Besides sialic acid binding mediated by the V-set domain, the C2-set domains of Sn display N-linked glycans that can act as ligands for other mammalian lectins such as the cysteine-rich domain of the mannose receptor and the macrophage galactose-type C-type lectin-1 (see Chapter 31). Although the biological significance of these interactions is uncertain, it is interesting that both lectins are found on dendritic cell populations that traffic into lymph nodes where Sn is abundantly expressed (Figure 32.3).

A role for Sn in interactions with pathogens has also been suggested. On the one hand, Sn can act as a phagocytic receptor for bacterial and protozoal pathogens such as Neisseria meningitidis and Trypanosoma cruzi, which coat themselves with Sias in an attempt to evade other forms of immune recognition. On the other hand, Sn expressed on alveolar macrophages can be “hijacked” as an endocytic receptor for the porcine reproductive and respiratory syndrome virus, which derives its envelope surface Sias from the mammalian cells from which it originally buds.

Sn-deficient mice appear essentially normal under specific pathogen-free conditions, with only subtle alterations in numbers of CD8 T cells and some subsets of B cells and reduced levels of circulating IgM. Granulocyte numbers appear normal in the bone marrow, in the blood, and following acute inflammation, suggesting that Sn–granulocyte interactions are not essential for maintenance of myelopoiesis. Interestingly, in mouse models of inherited neuropathy in which Sn-deficient mice were bred onto a P0 heterozygous background or proteolipid protein transgenic mice, reduced infiltration of CD8 T cells and macrophages was observed, accompanied by attenuated demyelination. In a mouse model of autoimmune uveoretinitis, Sn-deficient mice exhibited reduced inflammation, lowered T-cell proliferative responses, and a reduced time of onset to disease. Taken together, these findings suggest that Sn is important for the fine-tuning of adaptive immune responses, but the mechanisms remain to be established.

**CD22 (Siglec-2)**

CD22 was initially identified in 1985 as a developmentally regulated cell-surface glycoprotein on B cells. It is expressed at approximately the time of Ig gene rearrangement and is lost when mature B cells differentiate into plasma cells. CD22 was cloned in 1990 and was shown to have seven Ig-like domains. The intracellular region of CD22 has six tyrosine-based signaling motifs, four of which function as ITIMs.

CD22 is a well-established negative regulator of B-cell activation, making an important contribution toward the threshold for signaling via the B-cell receptor (BCR) complex. Following BCR cross-linking, CD22 is rapidly tyrosine-phosphorylated on its ITIMs by the Lyn tyrosine kinase. This leads to recruitment and activation of the SHP-1 tyrosine phosphatase and subsequent inhibition of downstream signaling mediated via the BCR. Besides SHP-1, the Ca++ pump PMCA4 is also recruited and activated and plays a key role in efflux of intracellular Ca++, which results in dampening of signals that depend on elevated intracellular Ca++ concentration. Although additional activatory
signaling molecules are recruited to the phosphorylated tyrosine motifs in CD22, the net phenotype of CD22-deficient mice is consistent with a primary role of CD22 in negative regulatory signaling, manifest by up-regulated MHC class II expression, enhanced B-cell turnover, reduced numbers of recirculating B cells in the bone marrow, reduced numbers of marginal zone B cells, and reduced anti-IgM-induced proliferation.

Of all the Siglecs, CD22 has the highest specificity for sialylated ligands, binding primarily to α2-6-linked Sias of the type Neu5Ac(Gc)α2-6Galβ1-4GlcNAc, which are common capping structures of many N-glycans. Additional specificity can be conferred by the nature of the Sia moiety: Neither hCD22 nor mCD22 binds 9-O-acetylated Sias; mCD22 has a strong preference for Neu5Gc over Neu5Ac, whereas hCD22 binds both of the latter forms. Binding of mCD22 to NeuGcα2-6Galβ1-4GlcNAc has a $K_d$ value of 250 μM at 37°C and displays very rapid dissociation kinetics. Recombinant soluble CD22 can precipitate a subset of glycoproteins from cell lysates including CD45, a major sialoprotein of T and B cells carrying up to 18 N-linked glycans. Detailed kinetic studies with a range of native and enzymatically derivatized glycoconjugates have established that binding of CD22 to ligands is dependent on Sia density and linkage and appears not significantly influenced by the nature of the glycan carrier.

In common with many Siglecs, the Sia-binding site of CD22 on B cells is “masked” by cis-interactions with α2-6-sialylated ligands. However, when a B cell contacts another cell expressing high levels of α2-6-sialylated ligands, CD22 can redistribute to the points of cell contact, suggesting that trans-cellular communication can occur under physiological conditions. This could be important for altering B-cell activation thresholds and may help to ensure that signaling through the B-cell IgM receptor can only occur in lymphoid tissues where CD22 α2-6-sialylated ligands are particularly abundant on both T cells and B cells. In addition, some evidence for “unmasking” of the CD22-binding site has been observed following B-cell activation in vitro, and the specialized subset of B1 marginal zone cells that respond to carbohydrate antigens independently of T cells have constitutively unmasked forms of CD22. This could be important for lowering their activation thresholds, allowing efficient antibody production to foreign carbohydrate antigens.

Mouse mutants have shed light on how the lectin function of CD22 regulates its immunomodulatory role in B cells. ST6Gal-I-deficient mice lack ligands for CD22 and their B cells exhibit an anergic phenotype, essentially the opposite of the phenotype observed with CD22-deficient mice, which have hyperactivated B cells and enhanced signaling via the BCR. Crossing the ST6Gal-I-deficient mice with CD22-deficient mice restores B-cell signaling function, suggesting that CD22 is essential for the reduced BCR signaling seen in CD22-ligand-deficient mice. CD22 is known to associate with the BCR and this still occurs in ST6Gal-I-deficient mice, as well as in B cells transfected with a non-Sia-binding mutant of CD22. This argues strongly for a conserved non-Sia-dependent association of CD22 with BCR. These results, together with biochemical data showing that CD22 is homomultimerized via protein and Sia interactions, suggest that CD22 at the cell surface is the major counterreceptor for itself, possibly to the exclusion of other cell surface sialylated glycoproteins such as CD45. Furthermore, CD22 can associate with BCR in distinct microdomains of the B-cell plasma membrane, and its potential association with clathrin-rich microdomains and recycling at the cell surface are important topographical issues that require more study in the future. Thus, the anergic phenotype of ST6Gal-I-deficient mice could be due to excessive interactions of BCR with CD22, leading to mislocalization and aberrant signaling. In this model, the role of α2-6-sialylation could be to promote CD22 homomultimerization and sequestration of CD22 away from the BCR (Figure 32.4). Complementary data have arisen from the use of mouse mutants in which the sialic acid–binding capacity of CD22 has been selectively inactivated, but conflicting results were obtained using potent Sia-based inhibitors of CD22. Overall, the somewhat inconsistent data on functions of CD22 Sia recognition may reflect the reality of the biological role of this interaction in “tuning” the B-cell response appropriately to a given circumstance.

**Myelin-associated Glycoprotein (Siglec-4)**

MAG was identified in 1972 as a minor constituent of central nervous system (CNS) and peripheral nervous system (PNS) myelin. Cloned in 1987, it has five Ig-like domains and is highly conserved in mammals. MAG has a clear-cut ortholog in fish and may represent a primordial Siglec that gave rise to the immune Siglecs via gene duplication and “exon shuffling.” Alternative transcripts of MAG (L-MAG and S-MAG) contain long (105 amino acids) or short (74
amino acids) cytoplasmic tails, respectively. The two isoforms are developmentally and anatomically regulated: Equivalent amounts are found in the adult CNS, whereas S-MAG predominates in the adult PNS. MAG is expressed by myelin-forming cells, oligodendrocytes in the CNS, and Schwann cells in the PNS. In mature myelinated axons, it is found on the innermost (periaxial) myelin wrap but not in the multilayers of compacted myelin. MAG has features that indicate a role in adhesion and signaling in axon–glia and/or glia–glia interactions. L-MAG can recruit the nonreceptor tyrosine kinase Fyn, the calcium-binding protein S100β, and the phospholipase Cγ, whereas S-MAG has been reported to bind to tubulin and microtubules, supporting a role for IT as a cell-adhesion molecule linking the axonal surface and the myelinating glial cell cytoskeleton.

MAG-deficient mice develop normal myelin, but defects in myelin and axons increase as animals age, indicating a role for MAG in the maintenance of myelin and myelinated axons, rather than in the process of myelination. MAG-null mice display late-onset progressive PNS and CNS axonal atrophy and increased “Wallerian” degeneration. Myelinated axons of MAG-null mice fail to show characteristic myelin-induced increases in neurofilament phosphorylation and axon diameter, indicating that MAG signaling is required for appropriate myelin–axon communication.

MAG also makes an important contribution to the inhibitory activity of myelin on axon outgrowth and repair, a major factor in poor recovery from nervous system injury. MAG extracted from myelin, as well as expressed forms of soluble and cell-surface MAG, inhibits neurite outgrowth from a wide variety of neuronal cell types in vitro.

Genetic and biochemical evidence indicates that gangliosides are important physiological ligands for MAG, mediating both myelin-axon stability and inhibition of axon outgrowth. Recombinant forms of MAG bind selectively to the abundant axonal gangliosides GD1α and GT1b, and they bind with higher affinity to the minor ganglioside GQ1bα (see Chapter 10). The phenotype of MAG-deficient mice is similar to that of mice lacking an N-acetylgalactosaminyleransferase (GalNAcT) required for synthesis of gangliosides. In addition, the disialyl T antigen (NeuAcα2-3Galβ1-3[NeuAcα2-6]GalNAc-R), a structure found on O-glycans and gangliosides, effectively inhibited the binding of soluble MAG to a target ligand and reversed MAG-dependent inhibition of axon outgrowth. Binding of soluble MAG to certain neurons is Sia-dependent and blocked with antibodies to gangliosides. Neurons from GalNAcT-null mice are less responsive to MAG, whereas MAG still inhibits neurite outgrowth from mice lacking the “β-series” gangliosides (lacking GT1b but expressing GD1α; see Chapter 10) due to a mutation of a sialyltransferase, GD3 synthetase. These findings suggest that gangliosides GD1α or GT1b both act as functional docking sites for MAG on neuronal cells.

MAG also binds specifically and with high affinity to a family of GPI-anchored proteins, the Nogo receptor (NgR) family. Gangliosides and NgRs may act independently or interactively as MAG receptors, linking MAG binding to axonal signaling in different neuronal cell types (Figure 32.5). In one model, a lipid-raft-associated signaling complex on the surface of neurons mediates MAG-dependent inhibition of axon outgrowth. Binding of MAG to NgRs (and perhaps gangliosides) is accompanied by movement of the p75 neurotrophin receptor into glycolipid-enriched membrane microdomains. The p75 is then cleaved by α- and γ-secretase, leading to activation of the small GTP-binding protein Rho A in a protein kinase C–dependent manner. GTP-bound Rho A then activates a Rho-A-dependent kinase leading to changes in actin filaments and microtubules, resulting in inhibition of axon outgrowth. It remains unresolved whether MAG binding to NgRs is Sia-dependent. One possibility is that MAG has two binding sites, one in the amino-terminal V-set domain, which mediates Sia-dependent binding, and another in domain 4 and/or 5, which binds a protein determinant on NgRs. Thus, MAG appears to mediate its effects on neurite outgrowth via protein–glycan and protein–protein interactions with gangliosides and NgR, converging on Rho-A activation and the control of the axon cytoskeleton.

**GENOMIC ORGANIZATION AND EVOLUTION OF CD33-RELATED SIGLECS**

Genes encoding most of this Siglec subfamily are clustered together on human chromosome 19q13.3-13.4 or the syntenic region of mouse chromosome 7. They include CD33 (Siglec-3) and Siglecs-5, -6, -7, -8, -9, -10, -11, -12, and -14 in humans and CD33, Siglecs-E, -F, -G, and -H in the mouse. Similar clusters are found in other primates and rodents. It is difficult to assign definitive orthologs between Siglecs in primates and rodents, resulting in the current
use of different nomenclatures. One reason for this is that most IgSF domains are encoded by exons with phase-1 splice junctions. This permits exon shuffling without disrupting the open reading frame, resulting in generation of species-restricted hybrid genes that are difficult to distinguish from similarly organized genes in other species. A second reason is that the Sia-binding sites in the V-set domains of the Siglecs appear to have been rapidly evolving, presumably to change their binding specificity in response to the rapid evolution of the endogenous host sialome (see Chapter 14). There is also evidence for gene conversion events between adjacent genes and pseudogenes within this cluster in a species-specific manner. Of particular interest is the finding that humans show many CD33rSiglec differences compared with their closest evolutionary cousins (the chimpanzees), more than the differences between mice and rats (which shared a common ancestor much earlier). For example, Siglec-13 is specifically deleted in humans, but present in chimpanzees and baboons.

**STRUCTURE, EXPRESSION, AND FUNCTIONS OF CD33-RELATED SIGLECS**

The CD33rSiglecs share sequence similarity and certain structural features, for example, the presence of a linker region encoded by a separate exon between domains 2 and 3. Most contain a membrane-proximal ITIM and a membrane-distal ITIM-like motif. Although the latter is similar to the ITSM (switch motif) found in other signaling receptors of the immune system, there is no evidence currently that it is essential for phosphatase recruitment or signaling functions. The ITIM recruits and activates both SHP-1 and SHP-2 and is thought to be important for negative inhibitory signaling and modulating CD33rSiglec-dependent adhesion. Most CD33rSiglecs are restricted in expression to the immune system. A shared property may therefore be to regulate leukocyte functions during inflammatory and immune responses, including cell proliferation, differentiation, activation, and survival, perhaps via recognition of sialic acids as “self.” In addition, CD33rSiglecs are actively endocytic and could be important in the clearance and/or antigen presentation functions of myeloid cells, especially when involving siałylated pathogens. Brief notes on the properties and putative functions of each of the CD33rSiglecs in humans and mice are provided below.

**CD33 (Siglec-3)**

CD33 was identified with monoclonal antibodies in 1983 as a marker of early human myeloid progenitors also found on mature monocytes and some macrophages and cloned in 1988. The later cloning of Sn and the recognition of Sia-binding by CD22 prompted experiments to investigate CD33’s Siglec activity. CD33 has some preference for α2-6-over α2-3-siałylated glycans. It was the first of the CD33rSiglecs to be characterized as an inhibitory receptor. Cross-linking of CD33 with the activating FcγRI was shown to reduce Ca++ signaling and CD33 was able to recruit and activate the inhibitory tyrosine phosphatases SHP-1 and SHP-2 (Figure 32.6). Mutation of the proximal ITIM prevented recruitment of these phosphatases and also resulted in increased Sia-dependent binding of RBC. CD33 is also rapidly phosphorylated on serine residues as a downstream consequence of protein kinase C activation, but the biological significance of this is not clear. Antibodies to CD33 have been reported to inhibit the proliferation of both normal and leukemic cell populations and the differentiation of DCs from bone marrow precursors, suggesting a role of CD33 in regulating hematopoiesis. The endocytic property of CD33 is currently being exploited in the treatment of acute myeloid leukemia using Gemtuzumab, a humanized anti-CD33 monoclonal antibody coupled to the toxic antibiotic calicheamicin. The endocytic pathway is as yet poorly characterized for any member of the CD33-related family, but it requires intact tyrosine motifs and appears to be via a clathrin-independent mechanism, in contrast to endocytosis mediated by CD22.

Prior to the discovery of the CD33rSiglec family, a murine ortholog of CD33 was isolated with two Ig-like domains (61% amino acid identity), yet with a cytoplasmic domain showing considerably less homology. Two alternatively spliced forms of mCD33 that differ in the cytoplasmic region have been described, but neither contains the typical ITIM found in most other CD33rSiglecs. This may explain the lack of a robust phenotype in CD33-null mice. Furthermore, mCD33 has a lysine residue in the transmembrane sequence and may therefore couple to the DAP-12 transmembrane adaptor, as shown recently for mouse Siglec-H and human Siglecs-14 and -15. In contrast to hCD33, mCD33 is expressed mainly on neutrophils rather than monocytes, which also suggests a nonconserved function of this receptor.
Siglec-5 (CD170) and Siglec-14

Siglec-5 contains four Ig domains and mediates Sia-dependent binding to α2-3Gal, α2-6Gal(NAc), and α2-8Sia linkages, indicating a potentially broad binding specificity for protein- and lipid-bound glycoconjugates. Siglec-5 is prominently expressed in the myeloid lineage, but at a later stage in differentiation than CD33. Rather than being lost from neutrophils on exit from marrow into the blood, Siglec-5 is retained and is also expressed on circulating monocytes and subsets of tissue macrophages. It seems to be the only Siglec in humans expressed on plasmacytoid DCs, a specialized cell type responsible for rapid production of type I interferons following viral infections. Furthermore, Siglec-5 can bind and internalize sialylated strains of *N. meningitidis*, raising the possibility that it could play a role in recognition of these pathogens in humans. Siglec-5 has been shown to function as an inhibitory receptor following co-cross-linking with the activating high-affinity IgεR in transfected rat basophilic leukemia cells.

Siglec-14 was recently characterized as a novel human Siglec with three Ig domains and no ITIM-like motifs (Figure 32.7). The first two Ig domains are greater than 99% identical to those of Siglec-5 with only one amino acid difference. In contrast, the third Ig domain of Siglec-14 is much less similar, and its transmembrane region contains an arginine residue that can associate with the DAP-12 ITAM-containing adaptor. This curious partial homology is explained by the finding that the Siglec-5 and Siglec-14 genes appear to be undergoing concerted evolution in multiple primate species, suggesting that these receptors may function as paired activating and inhibitory receptors. It should be noted that almost all antibodies currently available against Siglec-5 cross-react with Siglec-14, raising uncertainty about the expression patterns of Siglec-5 described above.

Siglec-6

Siglec-6 was cloned from a human placental cDNA library and also during a screen for proteins that bind leptin, a hormone that regulates body weight. It has three Ig-like domains and the typical arrangement of ITIM and ITIM-like motifs in its cytoplasmic tail. Siglec-6 binds dimeric leptin with a *Kₐ* of approximately 90 nM, which reflects an affinity for leptin that is tenfold weaker than the leptin receptor (Ob-R), yet tenfold stronger than the related Siglecs-3 and -5. These findings indicate that although it is unlikely to mediate leptin signaling, Siglec-6 could function as a leptin “sink” and contribute to regulation of leptin plasma concentration. Another unusual feature of Siglec-6 is its high expression in placenta, being localized to cytrophoblastic and syncytiotrophoblastic cells, where its levels seem to increase during the progress of labor and delivery. Lower levels are expressed on B cells. Siglec-6 does not have an obvious ortholog in mice, but one is present in the chimpanzee and baboon.

Siglecs-7, -8, and -9

These three SiglecS have three Ig domains, share a high degree of sequence similarity, and appear to have evolved by gene duplication from a three-domain ancestral Siglec. Siglec-7 is the major Siglec expressed by NK cells and functions as an inhibitory receptor when expressed naturally on these cells, both in antibody-directed killing assays and in assays using target cells overexpressing GD3, which is a Siglec-7 ligand. Siglec-7 also mediates selective interactions with sialylated lipooligosaccharides of the human pathogen, *Campylobacter jejuni*, suggesting a role in host–pathogen interactions. Siglec-9 is prominently expressed on neutrophils, monocytes, and to a much lesser extent on subsets of NK cells and CD8 T cells. In contrast, Siglec-8 is expressed on eosinophils, with weaker expression on basophils. The original Siglec-8 cDNA clone lacked the typical ITIM and ITIM-like motifs. However, subsequent analyses showed that this represents a minor alternatively spliced form and that a transcript encoding the longer form is more abundantly expressed in eosinophils.

Inhibition of cellular activation by Siglecs-7 and -9 can be demonstrated following co-cross-linking with ITAM-coupled activating FcRs (U937 and RBL cells) or following T-cell receptor engagement (in Jurkat cells). A novel proapoptotic function for Siglec-8 expressed by eosinophils was discovered following antibody-induced cross-linking. This depended on generation of reactive oxygen species and caspase activation and was paradoxically enhanced in the presence of cytokine “survival” factors such as GM-CSF and interleukin-5 (IL-5). Similar observations were made with Siglec-9 expressed on neutrophils. In addition to inducing apoptosis, ligation of Siglec-9 in the presence of GM-CSF (granulocyte-macrophage–colony-stimulating factor) also resulted in a caspase-independent nonapoptotic form.
of cell death. Autoantibodies to Siglecs-8 and -9 have been shown to be present in human serum, and their induction of granulocyte cell death may be linked to the anti-inflammatory properties of intravenous immunoglobulin, a pooled human serum preparation used to treat certain human autoimmune disorders. The role of the Siglec tyrosine-based motifs and SHP recruitment in triggering cell death are currently unknown.

Analyses of the glycan-binding specificities of these proteins revealed striking differences among Siglecs-7, -8, and -9. Siglec-8 was found to bind best to a single unique ligand, 6′-sulfo-SLeX, whose natural expression pattern is only partly known. In contrast, Siglec-9 preferred a related structure, 6-sulfo-SLeX, and Siglec-7 bound well to both forms.

The closest mouse relative to Siglecs-7, -8, and -9 is Siglec-E, which falls into the same evolutionary clade and shares about 70% sequence similarity with all three proteins. Siglec-E appears to exhibit a combination of features found in Siglec-7 and Siglec-9, being expressed on similar cell populations and exhibiting similar sugar-binding activity. Although there is no ortholog of Siglec-8 in mice, the four-Ig domain mouse Siglec-F is expressed in a similar way to Siglec-8 on eosinophils and has a similar glycan-binding preference. It therefore appears to have acquired the same functions through convergent evolution. Siglec-F-null mice show exaggerated eosinophilic responses in a lung allergy model, suggesting that its normal role is to dampen such responses. Interestingly, Siglec-F ligands in the airways and lung parenchyma were also up-regulated during allergic inflammation.

**Siglecs-10 and -11**

Siglec-10 has five Ig-like domains and displays an additional tyrosine-based motif in its cytoplasmic tail. It is expressed at relatively low levels on several cells of the immune system, including monocytes, eosinophils, and B cells. It is the only CD33-related human Siglec that has a clear-cut ortholog in mice, designated Siglec-G. This, combined with phylogeny analyses, suggests that Siglec-10 represents an ancestral CD33rSiglec that may have given rise to the other members of this subgroup via gene duplication, exon loss, and exon shuffling. Mice deficient in Siglec-G show a tenfold increase in numbers of a specialized subset of B lymphocytes, the B1a cells, that make natural antibodies and fast T cell–independent antibodies to some bacteria. In addition, the B1a cells show exaggerated Ca-fluxing following BCR cross-linking. Siglec-11 also has five Ig domains that are 90% identical to Siglec-10, but with much lower similarity in the transmembrane and cytoplasmic regions. Siglec-11 in humans appears to be a chimeric molecule that underwent gene conversion with an adjacent Siglec pseudogene. Despite the high sequence similarity in the extracellular region, the sugar-binding properties are distinct, with Siglec-10 binding to both α2-3- and α2-6-linked Sias and Siglec-11 binding only weakly to α2-8-linked Sias. Siglec-11 also exhibits different expression patterns, being absent from circulating leukocytes, but expressed widely on populations of tissue macrophages, including resident microglia in the brain, where high levels of α2-8-linked Sias are present on gangliosides. Interestingly, this microglial expression appears unique to humans.

**Siglec-15**

Siglec-15 has two Ig-like domains: a short cytoplasmic tail and a transmembrane domain containing a lysine residue that allows association with the activating adaptor proteins DAP-12 and DAP-10. Siglec-15 preferentially recognizes the Neu5Acα2-6GalNAcα-(Sialyl-Tn) structure and is expressed on macrophages and/or dendritic cells of human spleen and lymph nodes. While Siglec-15 has the potential to be an activating receptor, it does not have an inhibitory counterpart like Siglec-14. Siglec-15 has been conserved throughout vertebrate evolution, and it presumably plays a conserved, regulatory role in the immune system. As with Sialyl-Tn in a tumor marker, one suggested possibility is that it functions in tumor surveillance.

**NATURAL MUTATIONS OF THE “ESSENTIAL” ARGinine RESIDUE**

As mentioned earlier, an “essential” arginine residue in all of the known Siglecs is important for binding Sia-containing ligands. Surprisingly, this arginine is frequently mutated in nature, resulting in loss of binding ability. Examples of this include Siglec-12 in humans, Siglecs-5 and -14 in the chimpanzee, gorilla, and orangutan, Siglec-6 in the baboon, and Siglec-H in the rat. The common arginine codon (CGN, where N is any nucleotide) tends to be highly mutable because of the CpG sequence. However, the frequency with which such events occurs is surprising.
suggesting that it might be a natural mechanism to eliminate Sia binding of a given Siglec when such activity becomes inappropriate under changing evolutionary pressures without requiring a complete loss of the Siglec.

**HUMAN-SPECIFIC CHANGES IN SIGLEC BIOLOGY**

The ancestral condition of some hominin Siglecs (e.g., Siglec-7 and Siglec-9) appears to have been preferential binding to Neu5Gc, a Sia that was specifically lost in human evolution about 2–3 million years ago. The loss of Neu5Gc could thus have resulted in extensive Siglec unmasking, possibly leading to a state of heightened innate immune reactivity. Some human Siglecs have undergone an adjustment to allow increased Neu5Ac binding, and the question arises as to whether the adjustment is yet complete. Possibly as a consequence of this event, several Siglecs seem to have undergone human-specific changes in comparison to our great ape evolutionary cousins. For example, Sn seems to be expressed on most human macrophages, whereas only subsets of chimpanzee macrophages are positive for Sn. This may be related to the fact that Sn in humans has a strong binding preference for Neu5Ac over Neu5Gc, similar to that seen in other species. Human Siglec-5 and Siglec-14 appear to have undergone a restoration of the “essential” arginine residue needed for Sia recognition, which is mutated in chimpanzees, gorillas, and orangutans. As mentioned earlier, the gene encoding Siglec-11 has undergone a human-specific gene conversion, resulting in a new protein with altered binding properties and new expression in brain microglia. Siglec-12 has suffered a human-specific inactivation of the essential arginine residue in humans, with subsequent permanent pseudogenization by a frame-shift in some humans. Siglec-13 has undergone a human-specific gene deletion. Expression patterns of some Siglecs also appear to have undergone changes, with the placental expression of Siglec-6 being human-specific and a general suppression of all CD33rSiglecs on human T cells compared with the chimpanzee. The functional implications of these human-specific changes in Siglec biology for physiology and disease deserve further exploration.

**FURTHER READING**

FIGURE 32.1

Domain structures of the known Siglecs in humans and mice. There are two subgroups of Siglecs: One group contains sialoadhesin (Siglec-1), CD22 (Siglec-2), MAG (Siglec-4), and Siglec-15, and the other group contains CD33-related Siglecs. In humans, Siglec-12 has lost its arginine residue required for sialic acid binding and Siglec-13 is deleted. The plus sign indicates the presence of a charged residue in the transmembrane domain, which has been shown to interact with the immunoreceptor tyrosine-based activatory motif (ITAM)-containing adaptor proteins DAP12 and DAP10. (ITIM) Immunoreceptor tyrosine-based inhibitory motif.
FIGURE 32.2

Structural basis of Siglec binding to ligands. X-ray crystal structures of the V-set domains of sialoadhesin (Sn) (A) and Siglec-7 (B) are shown complexed with sialic acid. (C,D) Molecular details of the interactions of sialic acid with Sn and Siglec-7. (Figure prepared by Dr. Helen Attrill.)
FIGURE 32.3

Biological functions mediated by sialoadhesin: Interactions of sialoadhesin on macrophages with cells and pathogens. (Right) Red staining shows a ring of sialoadhesin expressed by macrophages in the marginal zone of the spleen and green staining shows Siglec-H on the plasmacytoid dendritic cells.
FIGURE 32.4

Proposed biological functions mediated by CD22: CD22 glycan-dependent homotypic interactions in equilibrium with CD22–BCR interactions. The actual situation seems to vary between different cell types and analysis conditions. (BCR) B-cell receptor; (Sia) sialic acid.
FIGURE 32.5

Proposed biological functions mediated by myelin-associated glycoprotein (MAG): Interactions between MAG and molecules of the axonal membrane lead to inhibition of neurite outgrowth. For a full explanation, see text. (NgR) Nogo receptor.
FIGURE 32.6

Proposed biological functions mediated by CD33-related Siglecs: A generic CD33-related Siglec is represented, showing the location of the immunoreceptor tyrosine-based inhibitory motif (ITIM) and the potential for inhibitory signaling.

Symbolic Representations of Common Monosaccharides and Linkages

- Galactose (Gal)
- N-Acetylgalactosamine (GalNAc)
- Galactosamine (GalN)
- Glucose (Glc)
- N-Acetylgalactosamine (GlcNAc)
- Glucosamine (GlcN)
- Mannose (Man)
- N-Acetylmannosamine (ManNAc)
- Mannosamine (ManN)
- Xylose (Xyl)
- N-Acetylneuraminic acid (Neu5Ac)
- N-Glycolyneuraminic acid (Neu5Gc)
- 2-Keto-3-deoxyglucuronic acid (Kdo)
- Fucose (Fuc)
- Glucuronic acid (GlcA)
- Iduronic acid (IdoA)
- Galacturonic acid (GalA)
- Mannuronic acid (ManA)

**Symbol Key:**

- Use letter designation inside symbol to specify if needed

FIGURE 32.7

Chromosomal organization of CD33-related Siglec clusters in some rodents and primates. Note that the locus marked as 5* is now known to encode Siglec-14 in primates. (Reprinted, with permission, from Angata et al. 2004. Proc. Natl. Acad. Sci. 101: 13251–13256.)

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Chapter 43

Glycans in Acquired Human Diseases

Essentials of Glycobiology 2nd edition
Chapter 43  Glycans in Acquired Human Diseases

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Given the diverse and ubiquitous presence of glycans on all cell surfaces, it is not surprising that several human disease conditions involve acquired (noninherited) changes in glycosylation and/or in the recognition of glycans. This chapter discusses some examples of these situations and considers the mechanisms of the changes seen, as well as the pathophysiological roles of glycans. Wherever relevant, the potential therapeutic significance of the information is mentioned. Details regarding some of these situations are covered elsewhere in the text. Glycosylation changes in cancer and pathologies resulting from inherited human genetic disorders are discussed separately in Chapters 44 and 42, respectively. It will be evident from the examples presented in this chapter that acquired changes in glycans and/or in their recognition have a significant role in a variety of human diseases. In some cases, the evidence remains circumstantial, and further work is needed to define whether the glycan changes have a primary role. In many of the situations, detailed knowledge of the nature of glycan–receptor interactions could result in improved diagnostic or therapeutic approaches.

CARDIOVASCULAR MEDICINE

Role of Selectins in Reperfusion Injury

A variety of common cardiovascular disorders (e.g., stroke, myocardial infarction, and hypovolemic shock) are characterized by a period of decreased or absent blood flow followed by a state of reperfusion, which occurs either by natural mechanisms or because blood flow has been restored by medical intervention. Despite rescue of the tissue from permanent anoxemic necrosis, the entry of leukocytes into the reperfused area can initiate a cascade of events that ultimately results in tissue damage (called reperfusion injury). P-Selectin on the activated endothelium in the reperfused area and/or L-selectin on leukocytes have vital roles in mediating the initial steps of this cascade (see Chapter 31). Substantial data in animal model systems indicate that blockade of this initial selectin-based recognition can ameliorate the subsequent tissue damage. A major goal of some pharmaceutical and biotechnology companies has been to make small-molecule inhibitors that can be used to achieve this blockade in human patients (see Chapters 49 and 50 regarding the synthesis of small glycan molecules designed to be selectin inhibitors). Interestingly, some forms of heparin currently used to effect anticoagulation under some of these conditions also have the ability to block P- and L-selectin at clinically tolerable doses.

Roles of Selectins, Glycosaminoglycans, and Sialic Acids in Atherosclerosis

High levels of low-density lipoprotein (LDL) cholesterol and decreased high-density lipoprotein (HDL) cholesterol are associated with an increased risk of atherosclerotic lesions in the large arteries, which are the major cause of heart attacks, strokes, and other serious diseases. The very earliest phase of the development of atherosclerotic lesions (the fatty streak) involves the entry of monocytes into the subendothelial regions of the blood vessels. There is evidence that this process involves the expression of P- and/or E-selectin on the endothelium, which recognizes P-selectin glycoprotein ligand-1 (PSGL-1) or sialyl-Lewis^x on circulating monocytes. Indeed, atherosclerotic lesions in atherosclerosis-prone mice showed delayed progression in a P-selectin-deficient background, and even slower progression occurs in a combined P- and E-selectin-deficient state. The induction of endothelial P-selectin expression may result from oxidized lipids that are present in LDL particles and/or the inflammatory process that occurs in the early atheromatous plaque. It remains to be seen whether it is feasible to intervene in this process, because early lesions probably develop very slowly and relatively early in life. The subsequent subendothelial retention of LDLS in the early plaque is thought to occur at least partly via their interactions with proteoglycans. The interaction is thought to cause irreversible structural alterations of LDL, potentiating oxidation and uptake by macrophages and smooth-muscle cells. At the molecular level, clusters of basic amino acids present in apolipoprotein B (the protein moiety of LDL) appear to bind the negatively charged glycosaminoglycans of proteoglycans. Meanwhile, heparan sulfate (HS) found in the liver may regulate the turnover of lipoprotein particles. Several reports also indicate a lowered overall sialylation of LDL in patients with coronary artery disease. The pathophysiological significance of this finding and the mechanism(s) involved remain unclear. One hypothesis is that the desialylated LDL is more prone to be taken up and incorporated into atheromatous plaques.

DERMATOLOGY

Role of Selectins in Inflammatory Skin Diseases
Several inflammatory skin diseases (e.g., atopic dermatitis and contact dermatitis) are characterized by the entry of leukocytes into the dermis, where they have a pathogenic role in recruiting other types of cells and in mediating tissue damage. These types of skin lesions are sometimes associated with the chronic persistent expression of E-selectin on the endothelial cells. Independent evidence indicates that E-selectin can recruit circulating lymphocytes carrying the cutaneous lymphocyte antigen (detected by the antibody HECA452), which appears to be a specific E-selectin ligand epitope carried on a subset of PSGL-1 molecules (see Chapter 31). There is also evidence that some T-helper-1 (Th1) lymphocytes can be recruited into the skin by virtue of their expression of the PSGL-1 ligand for P-selectin. Many of these observations have been made only in experimental models. The potential for therapeutic intervention in these selectin-mediated processes has not been fully pursued.

ENDOCRINOLOGY AND METABOLISM

Pathogenesis and Complications of Diabetes Mellitus

Diabetes mellitus is a disease of dysregulated glucose metabolism, resulting from relative or absolute lack of insulin action. It is accompanied by characteristic long-term vascular and neurologic complications. One mechanism appears related to the high levels of free glucose in body fluids, which cause acceleration of a well-known nonenzymatic process in which the open-chain (aldehyde) form of the glucose reacts randomly with lysine residues on various proteins, resulting in reversible Schiff bases. With time, some of these adducts undergo the irreversible Amadori rearrangement. These then undergo a series of “browning” (Maillard) reactions, which eventually progress to advanced glycation end products (AGE). The resulting protein cross-links can damage cellular functions, and such adducts can also be recognized by receptors, for example, the receptor for advanced glycation end products (RAGE) and the macrophage scavenger receptor, perhaps participating in the process of atherogenesis. A current view is that this is a normal process of aging, which is accelerated in the setting of the chronic persistent hyperglycemia of uncontrolled diabetes mellitus. It is important to differentiate mechanistically and semantically between this nonenzymatic glycation (or “glucosylation”) process and enzymatic glycosylation that takes place in the endoplasmic reticulum (ER), Golgi apparatus, and cytoplasm, utilizing glycosyltransferases and nucleotide sugar donors.

Another metabolic change of particular interest in diabetes mellitus is the increased production of UDP-GlcNAc caused by the conversion of excess glucose via the glucosamine:fructose aminotransferase (GFAT pathway). A current hypothesis is that this increase in cytoplasmic UDP-GlcNAc gives a secondary increase of O-GlcNAc levels on nuclear and cytoplasmic glycoproteins, secondarily altering the phosphorylation of the same proteins and their functions (see Chapter 18). Specific molecular mechanisms involving such altered O-GlcNAcylation have been defined in animal models for complications such as diabetic cardiomyopathy (increased O-GlcNAcylation of various nuclear proteins) and erectile dysfunction (O-GlcNAcylation of endothelial nitric oxide synthase). Interestingly, several of the cytoplasmic proteins involved in insulin receptor signaling and resulting nuclear transcription changes are themselves O-GlcNAcylated and are functionally altered in diabetes.

Nephropathy is a diabetic complication associated with high mortality. It begins with low levels of urinary albumin excretion (microalbuminuria), which progresses to frank macroalbuminuria. Ultimately, nephrotic syndrome and a concomitant decrease in glomerular filtration rate progress to end-stage renal disease. The proteinuria has been correlated with a reduction in the HS proteoglycan content of the glomerular basement membrane. The underlying mechanism may involve a reduction in HS synthesis by glomerular epithelial cells that may, in turn, be caused by the high glucose in the environment. One theory is that the resulting decrease in anionic change and loss of HS proteoglycan are thought to affect the porosity of the glomerular basement membrane. However, recent genetic evidence in mice questions this hypothesis. Interestingly, high glucose also mediates increased plasminogen activator inhibitor-1 (PAI-1) gene expression in renal glomerular mesangial cells, via O-GlcNAc-mediated alterations in Sp1 transcriptional activity.

GASTROENTEROLOGY

Role of Gut Epithelial Glycans in Gastrointestinal Infections

Many gastrointestinal pathogens interact with the gut mucosa via recognition of glycan structures (see Chapter 34). Prominent examples include cholera toxin (which binds GM1 ganglioside) and Helicobacter pylori, the causative agent of peptic ulcer disease and gastritis (which binds Lewis type glycans in the stomach mucosa). Consideration is now being given to using orally administered soluble glycan inhibitors to impede the attachment of such pathogens in the gut. In this regard, it is interesting that a time-honored treatment for peptic ulcer disease was a combination of antacids and milk (which contains large amounts of free sialyloligosaccharides). In addition, the variety
and high concentrations of free glycans found in human milk (especially in the early days after birth of the baby) are thought to impede the ability of gut pathogens to bind to the mucosa and initiate infections.

**Autosomal Dominant Polycystic Liver Disease**

Autosomal dominant polycystic liver disease is thought to arise by somatic mutations in individuals who already have a mutated allele in one of two different genes. One is *SEC63*, which facilitates recognition of proteins by ER chaperones. The other is *PRKCSH* (also known as hepatocystin or the β subunit of α-glucosidase II), which was previously described as a protein kinase C substrate (80K-H) and is localized on the cell surface, in intracellular vesicles, and in the ER. The products of both these genes are involved in the translocation, folding, and quality control of newly synthesized proteins. The cysts in the liver usually develop later in life, and outgrowth is restricted to the biliary epithelium, suggesting a specific impact on a regulator of their proliferation rather than a gross effect on all glycoproteins. The specific proteins affected have not been identified, but recent studies of genetically altered mice suggest that HS proteoglycans play a role.

**Heparan Sulfate Proteoglycans in the Pathogenesis of Protein-losing Enteropathy**

Protein-losing enteropathy (PLE) is defined as the enteric loss of plasma proteins, which become life-threatening. The cellular and molecular mechanisms of this disease are not well understood, but it develops in some patients with congenital disorder of glycosylation type Ib (CDG-Ib) and CDG-Ic (see Chapter 42) or as a complication months to years following Fontan surgery to correct congenital heart malformations in patients with normal N-glycosylation. PLE appears to result from a collision of genetic insufficiencies and environmental stress. Impaired N-glycosylation, increased proinflammatory cytokines, and increased venous pressure all synergize to create PLE. In each of these pathologies, HS is specifically absent from the basolateral surface of intestinal epithelial cells during the episodes, and it returns when PLE subsides. The inflammatory cytokines tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) both bind to HS, which may thus serve as a buffer. In vitro studies show that removal of HS from the basolateral surface increases cytokine signaling through their receptors, thereby loosening tight junctions that normally prevent protein leakage. Bacterial and viral infections increase these cytokines and often trigger PLE in patients. Some CDG-Ib and post-Fontan patients have increased venous pressure, which can down-regulate some of the genes involved in extracellular matrix biosynthesis. Pressure synergizes with the effects of cytokines and the localized loss of HS to create a downward spiral of disease. Traditional therapy for PLE includes treatment of the underlying conditions if possible, maintenance of nutritional state, and sometimes albumin infusions and steroid hormones or other anti-inflammatory drugs. The mother of a post-Fontan patient made the astute observation that her son’s PLE disappeared when he was given heparin injections as an anticoagulant prior to surgery. Later, in vitro studies showed that a few micrometers per milliliters of heparin or HS completely reverse the synergistic effects of epithelial cell HS loss and cytokine-induced breakdown of tight junctions. Nonanticoagulant heparin therapy may hold new promise for a variety of PLE patients.

**Changes in Sialic Acid O-Acetylation in Ulcerative Colitis**

Ulcerative colitis is an inflammatory disease typically affecting the superficial epithelial layer of the rectum and the distal colon. Although the primary cause of the disease is unknown, a large body of evidence suggests both genetic and environmental factors, and remissions and exacerbations are common. The sialic acids of the colonic mucosa, which are normally heavily O-acetylated, lose this modification in ulcerative colitis. Whether or not this is of pathogenic significance is uncertain, but these modifications normally do render the sialic acids more resistant to bacterial sialidases that are found in the gut. There have been conflicting claims about the efficacy of heparin treatments in improving the symptoms of this disease.

**HEMATOLOGY**

**Clinical Use of Heparin as an Anticoagulant**

Preparations of heparin (a highly sulfated form of HS; see Chapter 16) are routinely purified from animal tissues (particularly porcine intestines) and are used as a fast-acting and potent anticoagulant in a wide variety of diseases that involve thrombosis, in medical procedures such as dialysis, and in surgical procedures such as open heart surgery. As described in Chapter 16, the mechanism of anticoagulation is precise, involving a specifically sulfated heparin pentasaccharide that interacts with circulating antithrombin and markedly enhances its ability to inactivate coagulation factors Xa and IIa (thrombin). The use of “unfractioned heparin” is being partially supplanted by various forms of low-molecular-weight heparins that seem to be easier to use and are associated with fewer complications. One explanation is that
the unfractionated heparin effects on factor IIa require a long chain that interacts both with the antithrombin and with the IIa itself, in a tripartite complex. In contrast, the shorter chains found in low-molecular-weight heparins only facilitate antithrombin inactivation of factor Xa. Thus, low-molecular-weight heparins affect Xa but not IIa levels. Most recently, a synthetic pentasaccharide that binds and facilitates antithrombin inactivation of factor Xa has been introduced as an alternative to heparin. Although these improvements have been valuable, it must be kept in mind that the original unfractionated heparin has a variety of other biological effects besides anticoagulation. Thus, other beneficial effects of heparin, such as the blockade of P- and L-selectin, are being reduced or even eliminated during the course of the switch to the low-molecular-weight heparins and the synthetic pentasaccharide.

A rare but feared complication of heparin treatment is heparin-induced thrombocytopenia. The pathogenesis appears to involve the formation of complexes between heparin and platelet factor-4 and the generation of antibodies against the complexes. These antibodies in turn then deposit on platelets, causing their aggregation and loss from circulation. Somewhat paradoxically, this process results in exaggerated thromboses, rather than bleeding. The incidence of this complication appears to be lower with the use of low-molecular-weight heparins, and it may be absent with the use of the pure pentasaccharide.

**Hemolytic Transfusion Reactions**

The invention of blood transfusion uncovered the existence of the ABO blood group system, which is dictated by different alleles of an α-Gal(NAc) transferase (for details, see Chapter 13). These and other less prominent glycan antigens are responsible for many of the hemolytic transfusion reactions that can occur when errors are made in blood typing. Active attempts have recently been under way to generate “universal donor” red blood cells, via enzymatic conversion of blood group A and B antigens to the O state.

**Acquired Anticoagulation Due to Circulating Heparan Sulfate**

Occasionally, patients with diseases such as cirrhosis and hepatocellular carcinoma spontaneously secrete a circulating anticoagulant and have an unusual coagulation test profile that makes it appear as if the patient has been treated with heparin. The anticoagulant activity can be purified from the plasma and has been identified as an HS glycosaminoglycan. The precise source of secretion has not been defined, and therapy is often difficult unless the underlying disease can be corrected or the liver transplanted.

**Abnormal Glycosylation of Plasma Fibrinogen in Hepatoma and Liver Disorders**

Plasma fibrinogen is heavily sialylated and the sialic acids are involved in binding calcium. Certain genetic disorders of fibrinogen are known to be associated with altered glycosylation of its N-glycans, which causes altered function in clotting. Patients with hepatomas and other liver disorders can also sometimes manifest increased branching and/or number of N-glycans, resulting in an overall increase in sialic acid content. This can present clinically as a bleeding disorder associated with a prolonged thrombin time. Patients with congenital genetic disorders affecting N-glycan biosynthesis (see Chapter 42) can also have thrombotic or bleeding disorders that may be partly explained by altered glycosylation of plasma proteins and/or platelets involved in blood coagulation.

**Paroxysmal Nocturnal Hemoglobinuria**

Paroxysmal nocturnal hemoglobinuria (PNH) is an unusual form of acquired hemolytic anemia (excessive destruction of red blood cells) that usually appears in adults. The defect arises through a somatic mutation in bone marrow stem cells that causes the production of one or more abnormal clones. The defect is an inactivation of the single active copy of the PIGA gene, an X-linked locus involved in the first stage of biosynthesis of glycosylphosphatidylinositol (GPI) anchors (for details on GPI anchor biosynthesis, see Chapter 11). Although several blood cell types show abnormalities, the red cell defect is the most prominent, being characterized by an abnormal susceptibility to the action of complement. This is now known to be due to the lack of expression of certain GPI-anchored proteins, such as decay-accelerating factor, that normally down-regulate complement activation on “self” surfaces. However, hypercoagulability also occurs, presumably due to loss of GPI-anchored proteins on other cells, such as monocytes. Interestingly, many of these patients later develop either bone marrow failure (aplastic anemia) or acute leukemia. It is now known that most normal humans already have a tiny fraction of circulating cells with the PNH defect. These presumably represent the products of one or more bone marrow stem cells that develop this acquired defect because of a single hit on the active X chromosome but then did not become prominent contributors to the total pool of circulating red blood cells. In this scenario, the independent occurrence of a process damaging other stem cells allows the “unmasking” of the PNH defect.

**Paroxysmal Cold Hemoglobinuria**

Patients with this rare disorder have a cold-induced intravascular destruction of red cells (hemolysis), which appears to be caused by a circulating IgG antibody directed against the red cell P blood group system. The pathogenesis of this disorder is unknown, but it tends to occur in the setting of some viral infections and in syphilis. The IgG antibody is demonstrated by the so-called “Donath–Landsteiner test,” where the patient’s serum is mixed either with the patient’s own red cells or with those from a normal person and chilled to 4°C. Hemolysis occurs after warming back to 37°C.

**Cold Agglutinin Disease**

This disease is caused by autoimmune IgM antibodies directed against glycan epitopes on erythrocytes. High titers of IgM agglutinins are present in serum and are maximally active at 4°C. This IgM is presumed to bind to erythrocytes that are circulating in the cooled blood of peripheral regions of the body. The antibody fixes complement, which then destroys the cells when they reach warmer areas of the body. There are several variants of the syndrome. One affects young adults and follows infection with *Mycoplasma pneumoniae* or Epstein-Barr virus (infectious mononucleosis). This antibody is typically directed against the so-called “i” antigen (poly-N-acetylatedammine), is polyclonal, and is generally short-lived, disappearing when the infection subsides. Because *M. pneumoniae* is itself known to have a receptor that recognizes sialylated poly-N-acetylatedammine, it is hypothesized that the autoimmune antibody results from a mirror-image, anti-idiotypic reaction to the initial antibody directed against the mycoplasma’s binding site. An idiopathic variant of cold agglutinin disease affects older individuals, involves a monoclonal IgM, and can be a precursor or an accompaniment to a lymphoproliferative disease such as Waldenström’s macroglobulinemia, chronic lymphocytic leukemia, or other lymphomas. These antibodies are typically directed against the “I” antigen (β1-6-branched poly-N-acetylatedammine) present on erythrocytes. Some less common variants of cold agglutinin disease involve antibodies directed against sialylated N-acetylatedammines. In some patients on chronic hemodialysis, the syndrome occurs due to the formation of antibody directed against the sialylated blood group antigen N.

**Tn Polyagglutinability Syndrome**

Tn polyagglutinability syndrome is an acquired condition in which the blood cells made by the bone marrow express the Tn antigen (O-linked N-acetylatedammine, GalNAca-O-Ser/Thr) and sialyl-Tn (Sia2-6GalNAca-O-Ser/Thr), thus becoming susceptible to hemagglutination by the naturally occurring anti-Tn antibodies present in most normal human sera. The defect tends to be incomplete, with some circulating cells expressing the more complete sialylated tri- and tetrasaccharide O-glycans as well. These observations are best explained as an acquired stem-cell-based loss of expression of the O-glycan core-1 β1-3 galactosyltransferase activity (also called the T synthase). This in turn has now been explained by the acquired inactivation of Cosmc, a chaperone required for the biosynthesis of the T synthase. As with PNH, the existence of the *COSMC* gene on the X chromosome allows a single hit on the active X chromosome to cause a glycosylation defect in a single bone marrow stem cell. Patients with this syndrome show a wide range of symptoms. Some are picked up simply because the polyagglutinability of their red blood cells is detected when blood typing is done for a possible transfusion. Others have varying degrees of hemolytic anemia and/or decreases in other blood cell types. Some of these patients can subsequently progress into frank leukemia. It is unclear how the primary syndrome predisposes to the development of the malignancy. As with PNH, the possibility exists that an underlying bone marrow disorder simply allows the “unmasking” of preexisting minor stem cell clones with the defect. In keeping with this, the leukemic clones that arise later need not necessarily have the same defect.

**IMMUNOLOGY AND RHEUMATOLOGY**

**Changes in IgG Glycosylation in Rheumatoid Arthritis**

The IgG class of circulating immunoglobulins carry N-glycans, and those in the constant (CH2 or Fc) region of human IgG are reported to have several unusual properties. First, the glycans are buried between the folds of the two constant regions. Second, they are often sufficiently immobilized by carbohydrate–protein interactions that can be seen in the crystal structure of the protein (most glycans are not visible in crystal structures). Third, although processed into biantennary complex type-N glycans, they are hardly ever completed fully sialylated molecules. Instead, most of the molecules remain with one or two terminal β-linked galactose residues (so-called G1 and G2 molecules, respectively). It was previously noted that in patients with a chronic systemic disease called rheumatoid arthritis, a major fraction of the serum IgG molecules have decreased galactosylation of N-glycans, some carrying no galactose at all (so-called G0 molecules). The severity of the disease tends to correlate with the extent of the glycosylation change, and the spontaneous improvement that occurs during pregnancy is correlated with a restoration in galactosylation. One function attributed to the Fc N-glycans is to maintain the conformation of the Fc domains as well as the hinge regions. These structural features are necessary for effector functions such as complement binding and
Fc-dependent cytotoxicity. Nuclear magnetic resonance (NMR) studies have shown that the G0 N-glycans have an increased mobility resulting from the loss of interactions between the glycan and the Fc protein surface. Thus, it is thought that regions of the protein surface that are normally covered by the glycan are exposed in rheumatoid arthritis. In addition, some studies suggest that the more mobile G0 N-glycan may be recognized by the circulating mannos-β-galactosyltransferase activity in lymphocytes from patients with rheumatoid arthritis. It remains an open question whether the altered glycosylation of IgG has a primary pathogenic role in rheumatoid arthritis, because the appearance of G0 molecules is a general feature of other unrelated chronic granulomatous diseases, for example, Crohn’s disease and tuberculosis. Furthermore, the glycan change is also seen to a lesser extent in osteoarthritis, a form of chronic degenerative arthritis with a completely different pathogenesis. Overall, the change in IgG glycosylation in rheumatoid arthritis remains an interesting phenomenon whose precise significance and pathogenic role need further study.

**Secondary Changes in the O-Glycans of CD43 in Wiskott–Aldrich Syndrome**

This inherited genetic disease is characterized by skin eczema, altered cellular immune responses, and low platelet counts, symptoms that appear in childhood. Early studies suggested that the disease was associated with an absence of CD43 (also called leucosialin or sialophorin), the major O-glycosylated protein of lymphocytes. However, in retrospect, it is clear that this polypeptide is still expressed normally, but it has changed gel mobility because of markedly increased branching of O-glycans. Recent data indicate that the primary defect in this syndrome is not in glycosylation but in a transcription factor. However, the glycan changes seen in resting T cells of these patients are exactly the same as those that can be induced upon activation of normal T cells. Thus, it remains possible that some aspects of the immune disorders in this disease are due to a secondary change in glycosylation.

**INFECTION**

**Recognition of Glycans by Bacterial Adhesins, Toxins, and Viral Hemagglutinins**

A wide variety of pathogens initiate infection by specifically recognizing cell-surface glycans (see Chapter 34). In some instances, the differences in infection rates between individuals can be attributed to variations in the expression of the glycan target. For example, adhesion of certain pathogenic strains of *Escherichia coli* to cells in the urinary tract can be mediated by P fimbriae, involving a specific glycan receptor on the P blood group antigens. Infections do not occur in individuals who are P negative. P fimbriae also appear to be important in determining the propensity for bacterial bloodstream invasion from the kidney.

**Desialylation of Blood Cells by Circulating Microbial Sialidases during Infections**

Several microorganisms produce sialidases (classically called neuraminidases) that are involved in the pathogenesis of the diseases that they cause. In most instances, this enzyme remains localized to the site of infection. However, in some severe cases, for example, *Clostridium perfringens*-mediated gas gangrene, a sufficient amount of the sialidase is produced so that it can appear in the plasma. In this situation, the surface of circulating blood cells can become desialylated, resulting in enhanced clearance and anemia. The detection of the circulating sialidase has been proposed to have diagnostic and prognostic significance. Some cases of hemolytic-uremic syndrome are also associated with sialidase-producing *Streptococcus pneumoniae* infections. It is possible that blocking the sialidase with appropriate inhibitors could have therapeutic value in these situations.

**NEPHROLOGY**

**Loss of Glomerular Sialic Acids in Nephrotic Syndrome**

Nephrotic syndrome occurs when the kidney glomerulus fails to retain serum proteins during the initial filtration of plasma and these proteins then leak into the urine. The epithelial/endothelial mucin molecule called podocalyxin, which is present on the foot processes (pedicles) of glomerular podocytes, is thought to have a role in maintaining pore integrity and in excluding large molecules, such as proteins, from the glomerular filtrate. The sialic acid residues of podocalyxin molecules are believed to be critical in this process. Loss of
glomerular sialic acid is seen in spontaneous minimal-change renal disease in children and in the nephrotic syndrome that follows some bacterial infections. Several animal models seem to mimic this situation. Proteinuria and renal failure develop in a dose-dependent manner after a single inoculation of *Vibrio cholerae* sialidase, and this correlates with loss of sialic acids from the glomerulus. This was also accompanied by the effacement of foot processes and the apparent formation of tight junctions between podocytes. The anionic charge returned to endothelial and epithelial sites within two days of sialidase inoculation, but the foot process loss remained. Another model is termed aminonucleoside nephrosis, and it is induced in rats by injection of puromycin. Again, defective sialylation of a podocalyxin and glomerular glycosphingolipids has been detected in this model.

Changes in the O-Glycans in IgA Nephropathy

In humans, only IgA1 and IgD contain O-glycans in the hinge regions, whereas all immunoglobulin classes contain N-glycans in the Fc domain. Aggregation of the IgA1 molecule is thought to be involved in a form of nephrotic syndrome called IgA nephropathy. Studies of the O-glycans on serum IgA1 showed glycan truncations in the IgA nephropathy group compared with a negative control group. One of the functions of the IgA1 O-glycan chains is thought to be to stabilize the three-dimensional structure of the molecule. Studies of heat-induced aggregation support the notion that the altered glycosylation on the hinge region of IgA1 results in a loss of conformational stiffness, perhaps explaining the aggregation phenomenon. Removal of glycans from the IgA1 molecule also results in non-covalent self-aggregation and a significant increase in adhesion to extracellular matrix proteins. It is therefore suggested that the underglycosylation of the IgA1 molecule found in IgA nephropathy is involved in the noninmunologic glomerular accumulation of IgA1. The primary mechanism of underglycosylation remains unknown. A likely scenario is a defect in the *COSMC* gene, similar to that found in the Tn polyagglutinability syndrome (see above). The difference is that instead of affecting a bone marrow stem cell, the defect would involve a clone of B cells that specifically expresses IgA.

Heparan Sulfate in Systemic Lupus Erythematous

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by deposition of antigen-antibody complexes in various organs, especially the skin and the kidney. The precise initiating mechanisms of SLE are unknown, but it appears that the resulting pathology may involve both cytokines and HS. Amounts of HS are reduced on the glomerular basement membrane, and this was thought to result from masking of HS by complexes of nucleosomes and antinuclear antibodies, but the actual situation is likely to be more complex. Even though anti-double-stranded DNA antibodies are the hallmark of SLE, circulating antibodies to HS strongly correlate with disease activity. In some studies, HS injections into dogs induce SLE symptoms within several weeks. Elevated HS is found in the urine of SLE patients, especially in severe cases. SLE patients also have systemic elevated TNF-α. Anticytokine therapy against systemic and locally expressed cytokines suggests that blocking the proinflammatory cascade may be of significant value. Glomeruli have locally increased TNF-α, which acts to induce interleukin-1β (IL-1β) and IL-6. IFN-γ is thought to induce TNF-α, precipitating glomerulonephritis due to local inflammation. Some SLE patients also develop PLE, perhaps as a consequence of misplaced or degraded HS and elevated cytokines, creating the appropriate environment for PLE (see above).

NEUROLOGY AND PSYCHIATRY

Pathogenic Autoimmune Antibodies Directed against Neuronal Glycans

A variety of diseases are associated with circulating antibodies directed against specific glycan molecules that are enriched in the nervous system. These patients suffer from symptoms related to autoimmune neural damage. Such antibodies can arise via at least three distinct pathogenic mechanisms. In the first situation, patients with benign or malignant B-cell neoplasms (e.g., benign monoclonal gammapathy of unknown significance [MGUS], Waldenström’s macroglobulinaemia, and plasma cell myeloma) secrete monoclonal IgM or IgA antibodies that are highly specific for either ganglio-series gangliosides or, more commonly, for sulfated glucuronosyl glycans (the so-called HNK-1 epitope). These antibodies react with the glycolipid bearing this epitope 3-O-SO2-GlcAβ1-4Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer (3’sulfoglucuronosylparagloboside) and against the N-glycans on a variety of CNS glycoproteins (MAG, P0, L1, N-CAM) that bear the same terminal sequence (3-O-SO2-GlcA β1-4Galβ1-4GlcNAcβ1). The resulting peripheral demyelinating neuropathy can sometimes be more damaging than the primary disease itself. Therapy consists of attempts to treat the primary disease with chemotherapy or to remove the immunoglobulin by plasmapheresis. Both approaches are usually unsuccessful at lowering the immunoglobulin to a level sufficient to diminish the symptoms. The second situation is an immune reaction to the molecular mimicry of neural ganglioside structures by the lipoigosaccharides of bacteria such as *Campylobacter jejuni*. Following an intestinal infection with such organisms, circulating cross-
reacting antibodies against gangliosides such as GM1 and GQ1b appear in the plasma. These are typically associated with the onset of symptoms of a demyelinating neuropathy involving the peripheral and central nervous systems (the Guillain–Barré and Miller–Fisher syndromes, respectively). The third situation is a human-induced disease arising from recent attempts to treat patients with disease such as stroke using intravenous injections of mixed bovine brain gangliosides. Although some evidence exists that this treatment may benefit for the primary disease, several cases of Guillain–Barré syndrome have been reported as a likely side effect. One suggested scenario is that the presence of small amounts of gangliosides with the nonhuman sialic acid N-glycolyneuraminic acid facilitates the formation of antibodies that cross-react with ganglioside containing human sialic acid N-acetyleneuraminic acid.

**Role of Glycans in the Histopathology of Alzheimer’s Disease**

Alzheimer’s disease is a common primary degenerative dementia of humans, with an insidious onset and a progressive course. The ultimate diagnosis is made by postmortem histological examination of brain tissue, which shows characteristic amyloid plaques with neurofibrillary tangles that are associated with neuronal death. Two types of glycans have been implicated in the histopathogenesis of the lesions: O-GlcNAc and HS glycosaminoglycans. Paired helical filaments are the major component of the neurofibrillary tangle. These are primarily composed of the microtubule-associated protein Tau, which is present in a hyperphosphorylated state. This abnormally hyperphosphorylated Tau no longer binds microtubules and self-assembles to form the paired helical filaments that may contribute to neuronal death. Normal brain Tau is known to be multiply modified by Ser(Thr)-linked O-GlcNAc, the dynamic and abundant posttranslational modification that is often reciprocal to Ser(Thr) phosphorylation (see Chapter 18). The hypothesis currently being investigated is that site-specific or stoichiometric changes in O-GlcNAc addition may modulate Tau function and may also play a part in the formation of paired helical filaments by allowing excessive phosphorylation. The hyperphosphorylated Tau in Alzheimer’s disease brain is found in association with HS proteoglycans. Nonphosphorylated Tau isoforms with three microtubule-binding repeats form paired helical-like filaments under physiological conditions in vitro when incubated with HS. Heparin prevents Tau from binding to microtubules and promotes microtubule disassembly. These findings, together with previous evidence that heparin stimulates Tau phosphorylation by protein kinases, have been used to argue that sulfated glycosaminoglycans may be a critical factor in the formation of the neurofibrillary tangles. However, no significant difference was noted between the detailed structure of HS obtained from control brains and that from Alzheimer’s disease brains. Furthermore, the topological separation of Tau (which occurs in the cytoplasm) from glycosaminoglycans (which are extracellular) indicates that this physical association can only occur after cell death. On the other hand, HS proteoglycans may also have an important role in amyloid plaque deposition. Investigators have demonstrated high-affinity binding between HS proteoglycans and the amyloid precursor, as well as with the A4 peptide derived from the precursor. In addition, a specific vascular HS proteoglycan found in senile plaques bound with high affinity to two amyloid protein precursors. Overall, the data indicate that HS chains may have a significant role in the pathogenesis of the histological lesions.

**ONCOLOGY: ALTERED GLYCOSYLATION IN CANCER**

Altered glycosylation is a universal feature of cancer cells, but only certain specific glycan changes are frequently associated with tumors. These include (1) increased β1-6GlcNAc branching of N-glycans; (2) changes in the amount, linkage, and acetylation of sialic acids; (3) truncation of O-glycans, leading to expression of Tn and sialyl Tn antigens; (4) expression of the nonhuman sialic acid N-glycolyneuraminic acid, likely incorporated from dietary sources; (5) expression of sialylated Lewis structures and selectin ligands; (6) altered expression and enhanced shedding of glycosphingolipids; (7) increased expression of galectins and poly-N-acetyllactosamines; (8) altered expression of ABH(O) blood-group-related structures; (9) alterations in sulfation of glycosaminoglycans; (10) increased expression of hyaluronan; and (11) loss of expression of GPI lipid anchors. Some of these changes, for example, increased β1-6GlcNAc branching of N-glycans and expression of selectin ligands, have been shown to have pathophysiological significance in model tumor systems, and some are also targets for diagnostic and therapeutic approaches to cancer. For details regarding these topics, see Chapter 44.

**PULMONARY MEDICINE**

**Role of Selectins, Siglecs, and Mucins in Bronchial Asthma**

Asthma is a disease that is characterized by a hyperresponsiveness of the tracheobronchial tree to various stimuli, resulting in widespread narrowing of the airways, and it changes in severity, either spontaneously or as a result of therapy. The two dominant pathological features of asthma are airway wall inflammation and luminal obstruction of the airways by inflammatory exudates, consisting predominantly of mucins. Most cases are due to the presence of antigen-specific IgE antibodies, which then bind to mast cells as well as to basophils and
certain other cell types. Subsequently, antigen can cross-link adjacent IgE molecules, triggering an explosive release of vasoactive, bronchoactive, and chemotactic agents from mast cell granules into the extracellular milieu. Eosinophils also contribute to the pathogenesis of asthma in several ways, by synthesizing leukotrienes, stimulating histamine release from mast cells and basophils, providing a positive feedback loop, and releasing major basic protein, a granule-derived protein that has toxic effects on the respiratory epithelium. Underlying all this, it appears that CD4+ Th2 cells are responsible for orchestrating the responses of other cell types. Recent evidence indicates that the selectins are intimately involved in the recruitment of eosinophils and basophils (and possibly T lymphocytes) into the lung, raising the hope that small-molecule inhibitors of selectin function and/or heparin can be used to treat the early stages of an asthmatic attack. Likewise, chemokine interactions with HS are important in leukocyte trafficking. Recent evidence from Siglec-F knockout mice also suggests that the functionally equivalent human paralog Siglec-8 is a good target for reducing the contributions of eosinophils to the pathology (see Chapter 32). Finally, the large increase in mucus production is at least partly mediated by an up-regulation of synthesis of mucin polypeptides, under the influence of various cytokines that stimulate the goblet cells of the airway epithelium.

Role of Selectins in Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome is a serious pathophysiological process that is the final common pathway of lung injury arising from a variety of events, such as shock, trauma, or sepsis. It is characterized by diffuse pulmonary endothelial injury, progressing to pulmonary edema, which results from a marked increase in capillary permeability. Selectins and integrins help circulating neutrophils to adhere to the endothelium and release injurious oxidants, proteolytic enzymes, and arachidonic acid metabolites, resulting in endothelial cell dysfunction and destruction. The presence of many neutrophils and secretory products in bronchoalveolar lavage liquid emphasizes the critical role of the underlying inflammatory response. Again, the hope is that small molecule selectin inhibitors and/or the right kind of heparin can be used in the early stages of this syndrome, before it progresses to extensive lung damage and respiratory failure.

Altered Glycosylation of Epithelial Glycoproteins in Cystic Fibrosis

Cystic fibrosis is a very common genetic disorder caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR). This causes defective chloride conduction across the apical membrane of involved epithelial cells. Cystic fibrosis is associated with increased accumulation of viscous mucins in the pancreas, gut, and lungs, which leads to many of the symptoms of the disease. In the lung airways, there are known to be widespread increases in sialylation of secreted proteins and increases in the sulfation and fucosylation of mucus glycoproteins. One possible explanation is that the primary CFTR defect allows a higher Golgi pH, resulting in the abnormalities in glycosylation: however, there is currently some controversy about this conclusion. Curiously, the CFTR is mainly expressed within nonciliated epithelial cells, duct cells, and serous cells of the tubular glands, but it is not highly expressed in the goblet cells and mucous glands of the acinar cells, which are the cells that synthesize respiratory mucins. Thus, the CFTR mutation may indirectly affect mucin glycosylation through the generation of inflammatory responses. Another major cause of morbidity in the disease is the colonization of respiratory epithelium by an alginate-producing form of Pseudomonas aeruginosa. Certain glycolipids and mucin glycans have been suggested to be the Pseudomonas receptors that help to maintain the colonization. The changes in glycolipid and mucin glycosylation could enhance the production of potential binding targets for organ colonization. The presence of bacterial products is also a proinflammatory condition, since the bacterial capsular polysaccharides may activate Toll receptors and lead eventually to neutrophil accumulation and organ damage.

FURTHER READING

(Because of the wide range of topics covered in this chapter, it is not feasible to provide literature citations for all of them. Some examples are provided but the reader should consult references at the end of the other cited chapters.)


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