Mammalian glycosylation in immunity

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Abstract

Glycosylation produces a diverse and abundant repertoire of glycans, which are collectively known as the glycome. Glycans are one of the four fundamental macromolecular components of all cells, and are highly regulated in the immune system. Their diversity reflects their multiple biological functions that encompass ligands for proteinaceous of receptors known as lectins. Since the discovery that selectins and their glycan ligands are important for the regulation of leukocyte trafficking, it has been shown that additional features of the vertebrate immune system are also controlled by endogenous cellular glycosylation. This Review focuses on the emerging immunological roles of the mammalian glycome.

Glycosylation is the enzymatic process that produces glycosidic linkages of saccharides to other saccharides, proteins and lipids, and is probably as ancient as life itself. Unicellular and multicellular organisms depend on glycosylation to produce monomeric and multimeric glycan linkages that are essential for cell viability and normal function[1–4]. The resulting glycome encompasses a diverse and abundant repertoire of glycans, which are one of the four fundamental macromolecular components of all cells (together with nucleic acids, proteins and lipids) (FIG. 1). Glycans have important biological functions in protein maturation and turnover, cell adhesion and trafficking, and receptor binding and activation[5–8].

Glycosylation is prominent in the lumen of the endoplasmic reticulum (ER) and in the Golgi apparatus. The cellular repertoire of glycans that are produced by glycosylation in these organelles of the secretory pathway reflects the combinatorial expression of subsets of glycosyltransferase and glycosidase enzymes, of which there are more than 200 in the mammalian genome. The formation and breakdown of glycans are regulated at several levels in the cell. One of the mechanisms involves transcriptional regulation of the genes that encode these enzymes, but others include access to substrates and molecular interactions that alter enzyme localization in the lumen of the ER and Golgi[2,9,10]. Changes in the glycome can occur in response to environmental and genetic stimuli, and are frequently associated with the acquisition of altered cellular phenotypes[1–10].

Glycosylation also occurs among proteins in the cytoplasm and nucleus through the actions of the Ogt glycosyltransferase, which produces a reversible O-linked β-*N*-acetylglucosamine (*O*-GlcNAc) post-translational modification[11]. *O*-GlcNAc linkages are important in numerous

physiological processes and disease. In contrast to this intracellular glycosidic bond that is formed by Ogt, secretory glycosylation in the ER and Golgi produces a large structural repertoire, including oligomeric glycan linkages that are presented at the cell surface and in extracellular compartments. Intracellular glycosylation has been reviewed elsewhere and is not discussed further in this Review[12].

Glycosylation can substantially modify the structure and function of proteins by steric influences involving intermolecular and intramolecular interactions and can mediate the production of glycan ligands for lectins (FIG. 2). Lectins are proteins with glycan-binding activity that were first described in plants but subsequently have been found in all cells, from microorganisms to humans[13–15]. Lectin–glycan binding is a means of molecular recognition, which organisms can use to identify and decode the biological information that is present in their own cellular glycome as well as the glycomes of other organisms.

The extracellular positioning of the secretory glycome and its phylogenetic variation between organisms (BOX 1) allows glycans to be involved in pathogen–host interactions[16]. Glycan linkages of the mammalian host are an abundant and attractive target for pathogens to establish an infection through lectin binding. Mammals have developed a phyloglycomic recognition system, which includes a subset of Toll-like receptors (TLRs) and C-type lectins, to detect the glycans of lower organisms as a means of non-self discrimination and immunological activation[17]. These pattern-recognition receptors and their responses to non-vertebrate glycans are not considered further in this Review.

Box 1

The extracellular glycome is phylogenetically distinct
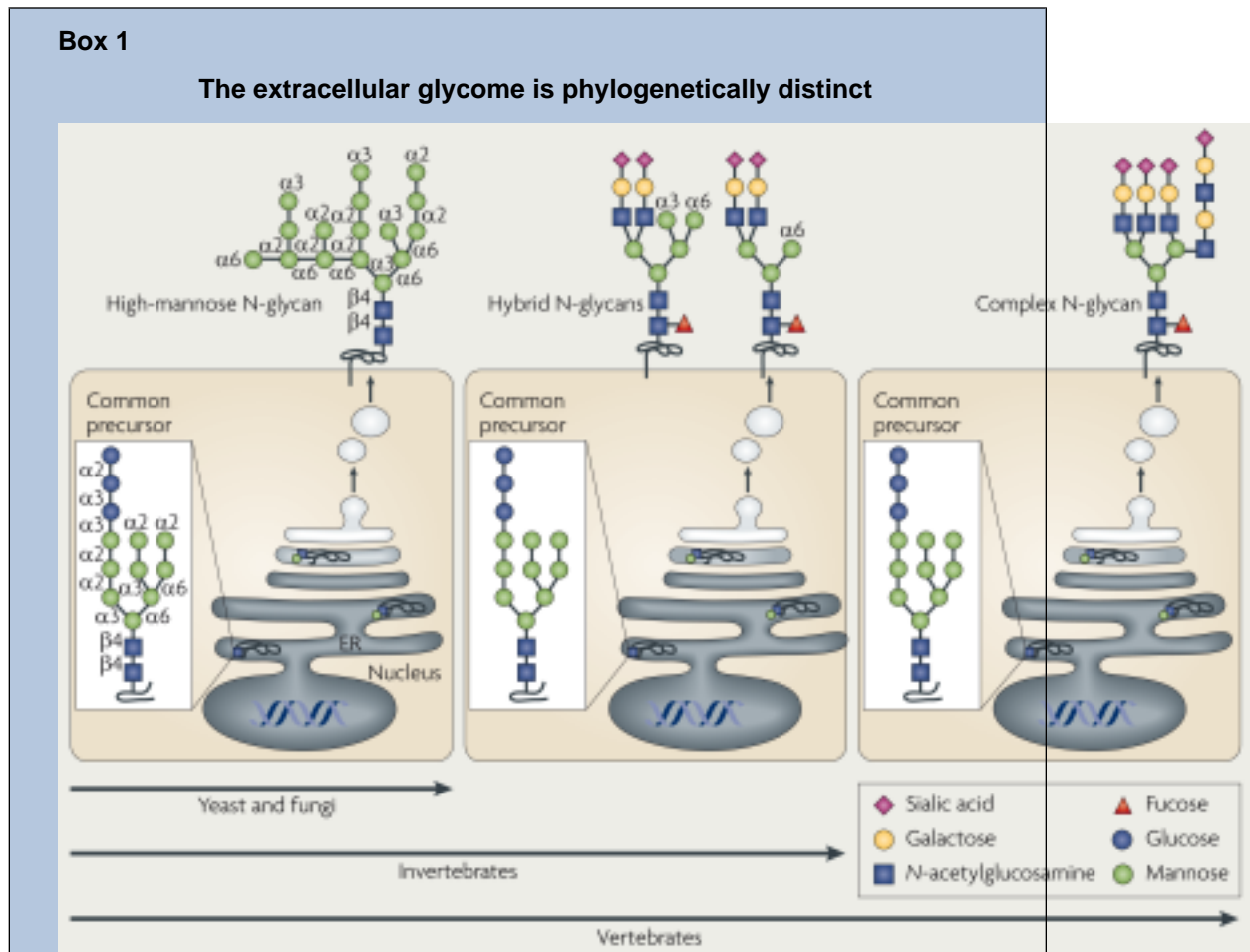
The glycome, which is the repertoire of glycans that bear glycosidic linkages, is produced by the enzymatic process of glycosylation. The glycome of a cell at any given time reflects the cell's 'portfolio' of functioning glycosyltransferases and glycan-modifying enzymes. The combinatorial and sequential function of glycosyltransferases and glycosidases in the secretory pathway contribute to the diversity of cellular glycans and provide a means for the regulation of glycan formation and turnover. In the lumen of the endoplasmic reticulum (ER) and Golgi apparatus, resident glycosyltransferases participate in the stepwise incorporation of saccharides into glycan chains and branches. As proteins and lipids transit through the secretory pathway, they are differentially modified by glycosyltransferases and glycosidases, which add and remodel glycan linkages, thereby promoting glycan diversification. Other enzymes, including sulphotransferases, can further modify these nascent glycan linkages. The resulting 'mature' glycans exist alone or attached to proteins and lipids, and are mainly transported to the cell surface and to extracellular compartments.

Glycan complexity has increased with evolution (see figure), such that there has been a decrease in the use of mannose linkages and the addition of new glycosidic bonds that bear different saccharides, including sialic acid. This is particularly evident when comparing the N-glycan biosynthetic pathway in lower eukaryotes (yeast and fungi), invertebrates and vertebrates. The increased complexity of the glycome through phylogeny has led to the formation of glycan-dependent epitopes that distinguish stem cells, different cell lineages and cellular activation states, and that can be diagnostic of inherited genetic diseases and prognostic in cancer[174–176].

The development and function of the mammalian immune system are associated with restructuring of the cellular glycome. The immune-cell glycome is altered during cell differentiation, activation and apoptosis, and these alterations are being linked to both homeostatic and disease mechanisms of immune-cell functions, including immune-cell trafficking and differentiation, antigen- and cytokine-receptor activation, auto-immunity, and the induction of leukocyte apoptosis[18–21]. This Review discusses the roles of mammalian secretory glycosylation in the immune system as established by *in vivo* studies that combine knowledge of the glycan biosynthetic pathways with genetic and cellular research approaches.

Cell trafficking

Of the immunological functions that have been ascribed to mammalian glycosylation, perhaps the best understood involves the glycan ligands of the lectins E-selectin, L-selectin and P-selectin[22,23]. The selectins and their ligands form a cell–cell adhesion system that operates mainly between leukocytes and endothelial cells. This system is essential for the tethering and rolling of circulating leukocytes on the vascular cell wall, which promotes subsequent firm binding by the integrins and the transmigration of leukocytes through the endothelium and into the surrounding tissue[24–26]. This mechanism of cell trafficking is responsible for the colonization of lymphoid organs by lymphocytes during development and allows leukocytes to migrate into lymphoid organs or inflamed tissues during an immune response. Each selectin binds a particular glycan ligand, such as sialyl-Lewis-X (sLe^x) for E- and P-selectin, and 6-sulpho-sLe^x for L-selectin. The binding interactions between selectins and their ligands can occur between many different cell types, including between lymphoid and non-lymphoid, as well as non-immune, cells[26,27].

Regulation of selectins and their ligands

Various cellular mechanisms regulate selectin function. Selectin expression, for example, is normally restricted to endothelial cells, platelets and leukocytes: E- and P-selectin expression is induced on the vascular endothelium during inflammation, P-selectin expression is also

induced on platelets and L-selectin is expressed on the surface of leukocytes[28]. In addition, expression of the glycoproteins that 'carry' (that is, are scaffolds for the construction of) the selectin ligands sLe[x] and 6-sulpho-sLe[x] can be similarly regulated, thereby altering the abundance and cellular localization of sLe[x] and 6-sulpho-sLe[x]. The glycoproteins that carry selectin ligands continue to be discovered and include P-selectin glycoprotein ligand 1 (PSGL1; also known as SELPG), E-selectin ligand 1 (ESL1), CD34, CD44, glycosylation-dependent cell-adhesion molecule 1 (GlyCAM1) and $\alpha_M\beta_2$-integrin [23,29,30]. Another mechanism that controls selectin function involves the combinatorial enzymatic activities of different Golgi-resident glycosyltransferases and sulphotransferases that contribute to the synthesis of sLe[x] and 6-sulpho-sLe[x]. These enzymes are tightly regulated in different cell types and can therefore contribute to the diverse functions of selectins in the immune system.

The synthesis of selectin ligands requires a set of enzymes, including fucosyltransferases, sialyltransferases, galactosyltransferases, glucosaminyltransferases and sulphotransferases, that act in a specific order on an underlying glycan chain as it transits through the Golgi apparatus. The expression of these enzymes can be regulated in response to intracellular signalling and transcription-factor induction[31–33]. The sLe[x] and 6-sulpho-sLe[x] glycans exist *in vivo* mainly on the O-glycan chains of glycoproteins, and their production depends on the expression of the fucosyltransferases Fut4 and Fut7. The absence of Fut7 or Fut4, and in particular of both enzymes together, markedly attenuates lymphocyte homing to peripheral lymph nodes and myeloid-cell trafficking in inflammation[34–36].

The $\alpha2,3$-sialyltransferases ST3Gal4 and ST3Gal6 seem to participate in the formation of selectin ligands *in vivo*. Studies of mice that lack ST3Gal4 have revealed a partial deficiency in selectin ligands, with a marked effect on leukocyte homing and trafficking in inflammation, which was not observed in mice that lack ST3Gal1, ST3Gal2 or ST3Gal3 (REFS [37–39]). The *in vivo* effect of ST3Gal6 deficiency has yet to be reported.

The contribution of O-glycans in carrying selectin ligands *in vivo* has been investigated in mice that lack core 2 *N*-acetylglucosaminyltransferase 1 (GCNT1) activity. GCNT1 is one of three glycosyltransferases that construct the core 2 O-glycan branch, which is a type of O-glycan structure that is commonly found on O-glycoproteins. GCNT1 deficiency strongly attenuated E- and P-selectin-mediated inflammation involving myeloid cells, but had relatively little effect on the lymphoid system, including the formation of secondary lymphoid organs and adaptive immune responses[40]. Subsequent studies of GCNT1-deficient mice detected a subtle but significant defect in the formation of L-selectin ligands in cells that form the high endothelial venules of some lymph nodes, which was associated with decreased kinetics of peripheral lymphocyte homing[41,42]. The finding that GCNT1 deficiency did not severely affect L-selectin ligands and lymphoid trafficking was addressed by the discovery of an endogenous β1,3-glucosaminyltransferase. In endothelial cells, this enzyme compensates for the lack of core 2 O-glycan branches and extends the core 1 O-glycan branch to provide a scaffold on which L-selectin ligands can also be produced[43]. Moreover, with loss of both this core-1-extension enzyme and GCNT1, L-selectin ligands were observed on N-glycan chains, which seem to contribute to a robust lymphocyte homing response in the absence of these two glycosyltransferases[44]. Compensation was also observed between the glycan sulphotransferases that are required for 6-sulpho-sLe[x] synthesis, namely GlcNAc6ST1 and GlcNAc6ST2. In contrast to deficiency of a single enzyme, deficiency of both sulphotransferases led to almost complete loss of L-selectin-ligand formation on the high endothe-lial venules of lymph nodes, together with a marked decrease in lymphocyte homing and immune function[45,46]. The different defects of innate and adaptive immune responses that are associated with these profiles of selectin-ligand deficiency reflect the combinatorial and regulated expression of glycosyltransferases and glycan sulphotransferases that construct the

E-, L- and P-selectin ligands. This modulation of ligand-expression profiles has progressed our understanding of the complexity of selectin function in the mammalian immune response.

Selectin-targeted therapy

Selectin-mediated binding promotes cell–cell adhesion that induces intracellular signalling and could therefore be exploited in therapeutic approaches. Once engaged by their cognate glycan ligands, the selectins recruit tyrosine kinases, such as FYN and spleen tyrosine kinase (SYK), to their cytoplasmic domains, thereby inducing intracellular tyrosine phosphorylation, activating mitogen-activated protein kinases and promoting integrin activation[47–50]. Pharmacological intervention that is focused on inhibiting the interactions between leukocytes and endothelial cells might therefore block the intracellular signalling pathways that are induced by selectin binding. In some disease contexts, selectin-mediated cellular trafficking might contribute to tumour-cell adhesion in metastasis and leukocyte infiltration in autoimmune disease. Indeed, blocking selectin binding or inhibiting the synthesis of selectin ligands can attenuate the pathogenesis of inflammatory diseases and decrease the tumorigenicity of cancer cells[51–55]. In other contexts, the induction of selectin ligands can be beneficial. Glycan remodelling of the mesenchymal stem-cell surface *in vitro* to express selectin ligands increased cell tropism for the bone marrow after transplantation and resulted in increased bone formation[56]. Pharmacological alteration of the production of selectin ligands and modulation of the binding of selectins *in vivo* are active areas of research and development that could ultimately lead to new treatments for the diseases of the immune system that are linked with leukocyte trafficking and activation.

Adaptive immunity and autoimmune disease

During their maturation, the antigen receptors of B and T cells are post-translationally modified with N- and O-glycan chains. The addition of different N-glycan linkages to the B-cell receptor (BCR) and T-cell receptor (TCR) by various glycosyltransferases in the Golgi apparatus seems to alter the molecular interactions at the cell surface that regulate receptor trafficking, association with other glycoproteins on the cell surface, signal transduction and receptor internalization by endocytosis. Moreover, glycans and endogenous multivalent lectins that bind to them might, in some cases, be engaged in a precise spatial and geometrically predictable array on the cell membrane (often termed a lattice) that could control glycoprotein function (see later).

TCR signalling

Glycosylation is involved in the cellular mechanisms that control the threshold of TCR activation, and can therefore have profound effects on the function of the adaptive immune system. The GnT5 glycosyltransferase is encoded by *Mgat5* (mannoside acetylglucosaminyl transferase 5) and initiates formation of the β1,6 N-glycan-branch structure on various glycoproteins, including the TCR. This N-glycan branch commonly includes polylactosamine, which consists of *N*-acetyllactosamine disaccharide repeats and is a ligand for various lectins of the galectin family[57]. In studies of mice that lack GnT5 function, TCR clustering is significantly increased, which coincides with a decreased threshold of T-cell activation (FIG. 3a). GnT5-deficient mice have increased delayed-type hypersensitivity responses, increased susceptibility to experimental autoimmune encephalomyelitis (EAE) and increased glomerulonephritis that is associated with immune-complex deposition[58]. The phenotype that is conferred by GnT5 deficiency also includes a TCR trafficking abnormality, and the resulting hyperimmune response might be a contributing factor in some human autoimmune diseases[59].

Endogenous lectins have been implicated in the formation of homotypic and heterotypic TCR interactions that modulate immunological signalling. Galectin-3 is chemically crosslinked with the TCR on naive resting T cells in a GnT5-dependent manner, and disruption of this interaction with glycan-ligand mimetics of galectin-3 also results in increased TCR clustering and a decreased threshold of activation[58]. By contrast, in post-activated mature cytotoxic T cells, a decrease in effector activity, such as occurs during anergy, can be reversed by *ex vivo* treatment with glycan-ligand mimetics of galectin-3. Decreased effector activity is associated with decreased co-localization of the TCR and CD8, and decreased binding of the T cells to HLA–peptide tetramers[60] (FIG. 3b). Studies of galectin deficiency in mice also indicate that galectin-1 modulates the threshold of TCR signalling during thymocyte development[61]. Interestingly, treatment of cells or mice with high concentrations of GlcNAc increases GnT5-mediated N-glycan branching and inhibits TCR activation and autoimmune responses in mouse models of EAE and type 1 diabetes[62]. This is thought to be the result of a higher concentration of the cellular donor UDP (uridine 5′-diphosphate)–GlcNAc in the Golgi apparatus, which might increase the capacity of GnT5 and other closely related glycosyltransferases to produce GlcNAc linkages that contribute to N-glycan-branch formation.

BCR signalling

The BCR forms complexes with immunoregulatory glycoproteins that modulate the threshold of BCR activation, including the lectin CD22 (also known as Siglec-2), a member of the Siglec (sialic-acid-binding immunoglobulin-like lectin) family[63] that is expressed exclusively on B cells. The structure of CD22 includes an extracellular lectin domain at the amino terminus of several immunoglobulin-like domains, and an intracellular immunoreceptor tyrosine-based inhibitory motif repeat, which has an inhibitory role as it recruits the phosphatase SHP1 (SRC-homology-2-domain-containing protein tyrosine phosphatase 1) and thereby dampens BCR signalling[64]. Synthesis of the glycan ligands for CD22 requires the ST6Gal1 sialyltransferase, which adds sialic acid in an α2,6 linkage to the underlying galactose residue of the *N*-acetyllactosamine disaccharide[65]. Although this glycan ligand for CD22 is present on many glycoproteins, in addition to CD22 itself, CD22 tends to bind to the glycan ligands of neighbouring CD22 molecules, thereby forming homotypic multimers [66]. In the absence of ligands for CD22 owing to ST6Gal1 deficiency, BCR activation and humoral immunity are markedly decreased, and this coincides with increased co-localization of the BCR with CD22, constitutive recruitment of SHP1 to CD22, decreased protein phosphotyrosine signalling, increased BCR trafficking to clathrin-enriched microdomains and increased BCR endocytosis[65,67,68]. The absence of both CD22 and ST6Gal1 restored normal BCR signalling, microdomain association and rate of endocytosis. Measurements of intracellular signal transduction in response to BCR stimulation in the absence of the ST6Gal1-dependent ligands of CD22 show dominance of negative signalling that suppresses B-cell activation. This negative signalling complex prevents the development of autoimmune disease and markedly improves survival in LYN-tyrosine-kinase-deficient mice[68]. These findings are consistent with a model of BCR function in which Siglec-dependent binding of CD22 to the BCR dictates the threshold of immunological activation (FIG. 4).

CD22 can bind ligands that are presented in *trans* and this might modulate glycoprotein complex assembly and its localization at the B-cell surface[177]. Moreover, the induced co-localization of CD22 with the BCR in *cis* seems to occur in a Siglec-independent manner that might involve protein–protein interactions that are mediated by the other extracellular (immunoglobulin-like) domains of CD22. Such a mechanism might explain how binding of dendritic cells to CD22 in a Siglec-independent manner inhibits B-cell proliferation[69]. The biology of CD22 is complex and there is potential for many Siglec-independent functions, some of which could be influenced by the Siglec-binding activity of CD22.

The glycan-dependent immune modulation that has been defined for ST6Gal1 in B cells and GnT5 in T cells indicates that crucial glycoprotein-selective functions can be modulated by single glycosyltransferase genes that contribute to the cellular mechanisms of antigen-receptor activation and the pathogenesis of autoimmune disease.

Antibody function

The secreted immunoglobulins that are produced following B-cell activation and immunoglobulin class switching are glycoproteins with characteristic glycan linkages[70]. The glycans of IgG are important for binding to Fc receptors and for other effector functions, including binding to complement component 1q (C1q) and complement activation[71]. Alterations of endogenous IgG glycosylation have been reported in various immune diseases, including rheumatoid arthritis and IgA nephropathy. In patients with rheumatoid arthritis, the N-glycans that are attached to IgG often lack terminal galactose and sialic-acid linkages and therefore bear exposed *N*-acetylglucosamine[72]. This terminal linkage is common to non-mammalian vertebrate species as well as various invertebrates and lower organisms, and is a ligand for some endogenous mammalian immune receptors and lectins that might promote inflammatory responses[73,74]. In patients with IgA nephropathy, the O-glycans that are attached to IgA are similarly truncated to expose *N*-acetylglucosamine, which correlates with antibody deposition in the inflamed kidney[75]. Deficiency of the β1,4GalT1 glycosyltransferase (one of several galactosyltransferases) recapitulates the pathology of IgA nephropathy in mice[76]. By contrast, the extent of IgG sialylation correlates with decreased inflammation following Fc receptor binding[77]. This seems to be due to the α2,6 sialic acids on IgG that are contributed by ST6Gal1 and are linked to the anti-inflammatory effects of intravenous IgG when used as a therapy for autoimmune diseases[78].

Cytokine receptors

The core 1,6 fucose linkage is found on most N-glycans owing to the activity of Fut8 fucosyltransferase, and deficiency of this enzyme results in an emphysema-like disease in mice. Remarkably, this syndrome was linked to impaired binding of the receptor for transforming growth factor β1 (TGFβ1) to its ligand, and could be attenuated by increasing the concentration of TGFβ1 (REFS [79,80]). An inherited or sporadic gene mutation that introduces new glycosylation sites into a protein can also produce receptors with ligand-binding defects[81]. In this study, an N-glycosylation site that was found at a new position in the protein sequence of the interferon-γ receptor 2 (IFNγR2) resulted in the formation of an N-glycan at this location that disrupted the ability of the receptor to bind its ligand. Such pathological changes to protein glycosylation represent another mechanism by which glycosylation can contribute to protein dysfunction and disease aetiology. It seems that glycosylation in the Golgi apparatus can in some cases directly affect the binding of immunomodulatory receptors to cognate ligand.

Innate immunity and autoimmune disease

Glycans might have been used to sense the presence of non-self since the beginning of multicellular life. Glycomic differences in phylogeny and between species can allow for this type of molecular discrimination between self and non-self, and thus glycan structures are activators of the innate immune system and pathogenic triggers for autoimmune diseases. Unlike new-world primates and other vertebrates, old-word primates lack the α1,3 galactosidase (α1,3Gal) galactose-terminated glycan branch linkage owing to a germline mutation of the α1,3 galactosyltransferase. This results in a substantial fraction of circulating immunoglobulins in old-world primates (including humans) that bind the α1,3Gal epitope and contribute to the hyperacute rejection of xenotransplants from other mammals[82]. Another emerging example concerns endogenous antibodies that are specific for the sialic-acid variant *N*-glycolylneuraminic acid (NeuGc). Humans seem to be unique in their lack of the enzyme

that converts *N*-acetylneuraminic acid (NeuAc) to NeuGc, and the consumption of NeuGc in the diet could lead to its incorporation into endogenous glycoproteins, resulting in the formation of NeuGc-specific antibodies[83]. Antibodies specific for glycans are also induced during human Tn syndrome, which is a haematological stem-cell disorder that is associated with thrombocytopaenia and leukocytopaenia and is caused by a mutation of *COSMC* (also known as C1GALT1C1). This gene normally encodes a protein with chaperone function that is required for β1,3 galactosyltransferase activity in O-glycan biosynthesis[84]. Mutation of *COSMC* leads to a truncated O-glycan structure on haematopoietic cells and immunoglobulins (known as the Tn antigen) that functions as an epitope for antibody production and cell lysis and might contribute to IgA nephropathy and Henoch–Schönlein purpura[84].

The aberrant synthesis of endogenous glycans can result in the exposure of cryptic epitopes that are perceived by the immune system as non-self and induce an inflammatory condition in the absence of infection that is linked to the pathogenesis of autoimmune disease. This explains how α-mannosidase-II (αM-II; also known as MAN2A1) deficiency causes an autoimmune syndrome in mice that is remarkably similar to systemic lupus erythematosus. Disease pathogenesis in αM-II deficiency is non-haematopoietic in origin (unlike other autoimmune disease models), is independent of both the adaptive immune system and C3 complement activation, can be mitigated by intravenous IgG administration and is linked to chronic activation of the innate immune system[85]. The block in N-glycan maturation in the Golgi that is caused by αM-II deficiency increases the expression of phylogenetically primitive hybrid N-glycans at the cell surface and among extracellular glycoproteins. The exposed mannose linkages of these primitive hybrid N-glycans on multiple glycoproteins are ligands for endogenous mannose-binding lectin receptors of the innate immune system, and this interaction results in the chronic induction of a pro-inflammatory response that can recruit and activate macrophages. The requirement for complex N-glycan branching to safeguard against this immunological recognition of cryptic mannose ligands reveals a distinct mechanism in the pathogenesis of autoimmune disease that can be termed epitope modification or neo-ligand formation. Mechanisms that impair N-glycan branching could therefore contribute to the pathogenesis of various autoimmune syndromes of presently unknown origin.

The mechanisms that establish and maintain immunological tolerance also involve lectin–glycan ligand interactions that suppress immune-cell activation. For example, galectin-1 is becoming increasingly important in the study of T-cell and dendritic-cell biology and in the pathogenesis of immunological diseases[86]. Galectins are involved in attenuating graft-versus-host disease, collagen-induced arthritis, type 1 diabetes and T-cell-mediated tumour rejection[18,86–91]. Galectin-1 seems to bridge innate and adaptive immunity in part by regulating the Fc receptor- and MHC-dependent immune functions of monocytes and macrophages through an extracellular-signal-regulated kinase (ERK) signalling pathway[91]. The immunomodulatory activity of galectin-1 extends to its involvement in fetal–maternal tolerance during pregnancy. Deficiency of galectin-1 increases the loss of fetuses that have been conceived by the mating of allogeneic mice. In addition, treatment of galectin-1-deficient mice with exogenous galectin-1 restores normal fecundity, and this coincides with the induction of tolerogenic dendritic cells and the proliferation of interleukin-10 (IL-10)-producing regulatory T cells[92].

Normal and abnormal catabolism of glycans can also provide signals of immunological importance. Hyaluronan is a glycan that consists of a disaccharide repeat that modulates innate immunity by interacting with TLRs. This glycan is synthesized as a linear chain unattached to either a protein or a lipid and its size seems to be important. In response to tissue injury, endogenous hyaluronidases cleave extracellular hyaluronan into small fragments that induce inflammatory gene expression by binding TLR2 and TLR4 (REFS [93,94]). Some pathogens express hyaluronidase as a virulence factor that may cleave hyaluronan into fragments that do

not bind TLRs and thereby subvert the immune response. The enzymatic regulation of hyaluronan synthesis and degradation is increasingly recognized as having an important role in innate immunity and the response to infection[95].

Immune-cell differentiation

Glycoproteins, such as antigen receptors and MHC molecules, are important regulators of immune-cell differentiation and must be properly folded to function. Without correct folding and maturation in the ER and Golgi apparatus, the expression of improperly folded and dysfunctional glycoproteins on the cell surface and in extracellular compartments can cause defects in the signalling pathways that promote immune-cell differentiation. In addition, acquisition of secondary determinants of the differentiated immune-cell phenotype can also be controlled by glycan-dependent mechanisms. For example, glycosaminoglycan sulphation by the sulphotransferase NDST2 (*N*-deacetylase/*N*-sulphotransferase (heparan glucosaminyl) 2) promotes the development of mast-cell secretory granules, which are essential for the function of these immune cells[96,97].

T-cell ontogeny and endogenous glycans

The range and structures of peptides that are presented by MHC molecules could be influenced by glycan linkages on native glycoproteins and thus could contribute to the immunological definition of self[98]. Although peptides that are produced in the cell for presentation on MHC class I molecules are typically deglycosylated during proteolysis in the proteasome, some glycan linkages persist on various peptides and are recognized by cytotoxic T cells[99]. Peptides that arise from external sources can retain a broader range of glycan linkages, which might influence peptide proteolysis and loading on MHC class II molecules for binding by αβ TCRs. For example, some TCRs from T cells that have been isolated from mice with collagen-induced arthritis recognize an MHC class II-associated peptide of type II collagen that contains galactose linked to hydroxylysine residues of collagen[101]. Fate decisions in thymic T-cell development can be modulated by galectin-1, which promotes negative selection through ERK signalling[100]. Altered glycosylation in glycosyltransferase-deficient animals, for example, may influence the formation of the TCR repertoire and thereby have an effect in immunity. The repertoire of T cells in an intact mammal, which is determined by self-antigen presentation, might therefore reflect, in part, the portfolio of endogenous glycopeptides and their presentation by MHC molecules. This possibility could also involve intracellular *O*-GlcNAc glycan linkages that are attached to many cytoplasmic and nuclear proteins[102]. Similarly, glycolipids might be involved in development of the natural killer T (NKT)-cell lineage; the presentation of an endogenous glycolipid structure by the CD1 molecule might promote the development of a functional NKT-cell population[103].

T-cell repertoire development

Expression of the glycoproteins CD4 and CD8 as TCR co-receptors confers MHC class II and class I restriction of TCR activation, respectively, during T-cell development in the thymus. The sialylation state of glycans of the CD8–α β TCR heterodimer affects co-receptor binding to MHC class I molecules. A low level of O-glycoprotein sialylation in CD4+CD8+ thymocytes is associated with binding to MHC class I tetramers, whereas the increased abundance of sialic-acid linkages on differentiated CD8+ thymic T cells decreases the binding avidity of CD8 for MHC class I molecules, thereby decreasing T-cell adhesion to MHC class I tetramers[104–106]. This decrease in binding to MHC class I molecules is in part mediated by the sialic acids that are produced by the ST3Gal1 sialyltransferase, as deficiency of this enzyme in mice increases the binding of MHC tetramers to single-positive CD8+ thymic T cells and alters the repertoire of the TCR β-chain variable region (Vβ)[105]. This mechanism seems to involve a change in the CD8–αβ TCR heterodimer conformation that promotes MHC class I binding in the presence

of unsialylated core 1 O-glycans, which lack the steric hindrance and negative charge that are associated with sialic acid[105,107]. However, another study that used a different MHC–peptide complex showed no significant change in tetramer binding of ST3Gal1-deficient CD8^{+} thymic T cells[108]. The reasons for this discrepancy are not clear, but they might relate to the intrinsic binding affinities of the TCR transgenes and MHC tetramer probes that were used in each study. Nevertheless, the expression and deficiency of ST3Gal1 alter the TCR Vβ repertoire in an opposing manner, which indicates that ST3Gal1 has a role in the MHC-restricted thymic differentiation of CD8^{+} T cells[105,109].

Notch ligands and immune-cell development

The binding of <u>Notch</u> ligands to Notch receptors provides another example of how glycosyltransferases can influence intracellular signal transduction and thereby govern the development and function of immune cells. Notch signalling, which controls cell-fate determination in many organisms, dictates lineage commitment in the mammalian lymphoid system and has recently been shown to be involved in myelopoiesis[110]. The closely related Fringe glycosyltransferases (Manic, Radical and Lunatic in the mammalian genome) were discovered as genes that establish cell-boundary fields in embryogenesis and were later found to intersect with the functions of Notch receptors in early development[111–115]. The Fringe glycosyltransferases are β1,3 *N*-acetylglucosaminyltransferases that operate on the O-linked fucose glycans. These glycans are found in the extracellular epidermal growth factor (EGF)-like repeats of Notch receptors in the secretory pathway and control cell-surface Notch signalling[116,117]. The O-fucose linkage that is the substrate of the Fringe glycosyltransferases is first generated by Pofut1 (protein *O*-fucosyltransferase). The glycan products of the Fringe glycosyltransferases are then substrates of β1,3 galacto-syltransferases. These glycans provide a physiological mechanism to modulate Notch binding and activation by the extracellular glycoprotein ligands Jagged/Serrate and Delta[118–123]. In addition to generating the O-fucose linkage that is the substrate of the Fringe glycosyltransferases, Pofut1 provides a chaperone activity that is independent of its glycosyltransferase activity and promotes Notch maturation[124]. More recently, Notch signalling has been shown to be also influenced by the Rumi glycosyltransferase, which generates O-glucose linkages on the EGF-like repeats of Notch[125,126].

Signalling through the Notch axis has profound effects on lymphocyte development and activation in mammals. For example, a constitutively active form of Notch can promote the development of CD8^{+} T cells in the absence of MHC class I molecules[127]. The effect of Fringe glycosyltransferases on lymphoid-cell fate includes the suppression of B-cell development from lymphoid progenitors in the thymus[128]. However, Fringe activity promotes increased Notch binding to Delta-like ligands, which can lead to a block in T-cell development. This might be due to an effect on the access of progenitor cells to intrathymic niches or a direct effect on the induction of T-cell differentiation signals[129,130]. Moreover, Notch activation leads to the formation of marginal-zone B cells in the spleen and promotes immunoglobulin secretion by a signalling mechanism that involves Notch binding to the Delta-like 1 ligand[131–133]. Dendritic- and myeloid-cell differentiation also seem to be modulated by Notch signalling in response to the binding of Notch ligands, and the activation of NK-cell cytotoxicity is promoted by the Notch ligand Jagged2 (REFS [134,135]). Immune responses can also be inhibited by Notch binding to Fringe-dependent glycan ligands; for example, Delta-like-1 ligand seems to inhibit Notch activation, which skews the outcome in airway inflammation from a T helper 2 (T$_H$2)- to T$_H$1-cell response[136]. The functional relationships between Notch receptors and their glycan ligands are only partly understood, but already a remarkable complexity in glycan-mediated and Notch-dependent immune-cell differentiation is indicated.

T$_H$-cell polarization

Activated CD4$^+$ effector T cells can differentiate into distinct subsets (T$_H$1, T$_H$2, T$_H$17 and regulatory T cells), each of which produces a unique profile of cytokines that differentially govern immune responses to various stimuli. It seems that mammalian glycosylation has a role in the emergence and phenotypic profiles of these T$_H$-cell subsets, in part by its effect on the production of ligands of various lectins. The Delta-like 1 and Delta-like 4 glycan ligands of Notch promote T$_H$1-cell differentiation of CD4$^+$ T cells in part by suppressing IL-4-mediated signalling, which would otherwise induce T$_H$2-cell differentiation and promote T$_H$2-cell immune responses[137,138]. By contrast, the GnT5 glycosyltransferase promotes T$_H$2-cell immune responses by inducing differentiation signals that inhibit IFNγ production[139]. The induced deficiency of galectin-1 restores the T$_H$1–T$_H$2-cell balance of T-cell infiltrates in classical Hodgkin's lymphoma, apparently by modulating the secretion of T$_H$2-cell cytokines and the proliferation of regulatory T cells[140]. Differential glycosylation of T$_H$-cell subsets has been linked with their susceptibility to death by galectin-induced apoptosis, which provides an explanation for the increased T$_H$1- and T$_H$17-cell responses that have been observed in galectin-1-deficient mice[141]. A related mechanism might operate during parasitic infection by which helminth glycans skew CD4$^+$ T-cell immune responses towards the T$_H$2-cell phenotype[142].

Apoptosis

Cell death by apoptosis is an important mechanism of immune-system homeostasis during lymphoid development and in the clearance of effector cells after immune activation[143]. Mammalian glycosylation contributes to this process by generating ligands for endogenous lectins, including galectins[57,144,145]. These lectins bind to glycans that contain the disaccharide lactose and to polylactosamine, which are present in some cellular glycoproteins and glycolipids.

The T-cell lineage is sensitive to both the endogenous expression and exogenous administration of galectin, and most studies have examined the effects of galectin-1 and galectin-3. Exogenously administered galectin-1 was first reported to induce apoptosis in thymocytes, T-cell lines and activated T cells[146]. The *in vitro* methodology used was thought to contribute in part to this result, as a subsequent study reported that activated T cells do not normally undergo apoptosis in response to galectin-1, but become apoptotic after administration of galectin-3 (REFS [147,148]). In multiple cell types, including T cells and neutrophils, binding of galectins -1, -2, -3, -4 or -8 to cellular glycans was shown to induce phosphatidyl-serine exposure on the outer leaflet of the plasma membrane, which is associated with cellular apoptosis with phagocytic uptake *in vivo*, and normal cell viability was retained *in vitro*[147−150]. Galectins bind to glycan ligands that are present on various glycoproteins, such as CD2, CD3, CD7 and CD45, thereby forming glycoprotein complexes that induce intracellular signalling cascades and inhibit IFNγ and IL-2 production and IL-2-mediated signalling[148,151−154]. Among the glycan ligands of the galectins, the polylactosamine moieties that are found in O-glycans are sometimes present on the core 2 branch. T-lymphoma cells that are deficient in core 2 O-glycans as a result of *GCNT1* mutation or haploinsufficiency are resistant to galectin-1-induced apoptosis[155]. In addition, some galectins seem to oppose the functions of others. Galectin-3 binds different glycans and glycoproteins to those that are recognized by galectin-1, and galectin-3 can inhibit galectin-1- but not galectin-9-mediated apoptosis of some human T-cell lines. By contrast, galectin-9 induces T-cell apoptosis that can be inhibited by B-cell lymphoma 2 (BCL-2) (REFS [156−158]).

The ST3Gal1 sialyltransferase seems to control the largest and most synchronous apoptotic response in adult mammals, that of eliminating the increase in cytotoxic CD8$^+$ T cells that results from an immune response to infection by various microorganisms and viruses. More

than 90% of the clonally expanded CD8^{+} T-cell population must be rapidly cleared by apoptosis during the resolution of the immune response so that the body is not overcome by lymphocytosis. ST3Gal1 is involved in this process by linking sialic acid in an α2,3 linkage to the core 1 O-glycan of one or more O-glycoproteins. Attenuation of ST3Gal1 function by a post-translational mechanism occurs during the resolution phase of the immune response, and the resulting unsialylated core 1 O-glycan structure is linked to the onset of post-activated CD8^{+} T-cell apoptosis by a caspase-dependent mechanism that seems to dominate the effects of the intracellular apoptotic modulators BCL-2 and BIM[109,159]. Remodelling of the glycome in activated CD8^{+} T cells by downmodulation of ST3Gal1 function induces a signalling pathway that ensures apoptotic cell death in the absence of TCR stimulation and of factors that induce memory-cell differentiation. The lectin (or lectins) that induces post-activated CD8^{+} T-cell apoptosis *in vivo*, and the glycoprotein counter-receptor (or receptors) that transmits the apoptotic signal following binding to the unsialylated core 1 O-glycan ligand, are yet to be identified.

Summary and future directions

A rapid expansion of our knowledge of the immunological roles of mammalian glycosylation has resulted from interdisciplinary studies that combine biochemical, enzymatic, structural and genetic techniques. Establishing the relationships between glycan linkages, the enzymes that produce them, and the glycoproteins that are modulated by them remains at the forefront of research (TABLE 1). Studies that bridge immunology and glycobiology, and which link the enzymes of glycosylation with lectin recognition, will continue to provide new insights into the immune system and will reveal new approaches to therapeutic intervention in disease. Studies of glycosylation by the ST3Gal4 sialyltransferase, for example, recently revealed that the Ashwell–Morell receptor of hepatocytes — the main lectin expressed by hepatocytes — mitigates the lethal coagulopathy of sepsis[160], exposing an unexpected pathogen–host relationship that might reflect an ancient arm of the innate immune system.

The mechanisms by which glycans and lectins contribute to immunity are only now beginning to be understood. Among the potential mechanisms, the nature of multivalent lectin–ligand binding as observed *in vitro* indicates that higher-order structures might exist among glycoproteins and lectins at the cell surface[161]. Further study could resolve whether galectins normally operate *in vivo* to construct a repetitive and spatially precise ultrastructure (a lattice) that thereby modulates the presentation, interaction, function and turnover of glycoproteins[161−166]. There is increasing evidence that mammalian glycosylation controls the rate of receptor endocytosis as a means to modulate cellular responses through the interaction of lectins and their ligands[167−169]. It seems possible that what is presently considered basal protein-turnover activity at the cell surface is instead a highly orchestrated process that controls glycoprotein fate and function. Furthermore, it remains probable that more lectins without canonical lectin-like domains exist, which are identifiable as proteins that bind their targets and function in a glycan-dependent manner. It follows that glycan-dependent regulation of glycoprotein and glycolipid interactions might contribute in many contexts and in presently unknown ways to the mechanisms that govern the immune responses of cells to various extracellular stimuli.

The evolutionary success of the selectins and their glycan ligands in regulating immune function by controlling cell–cell adhesion and signalling makes it unlikely that they are the only lectin–ligand system that has evolved to direct immune-cell trafficking. Other glycan structures, such as Lewis X and Lewis Y, for example, have recently been found to participate in the cell–cell recognition that modulates dendritic-cell adhesion and signalling[170,171]. Moreover, hyaluronan has been recently found to modulate neutrophil adhesion in liver sinusoids[172]. The extent to which glycosylation contributes to immune-cell recognition is

uncertain, but the regulated changes that occur in glycan formation during cell maturation and activation are profound and may alter the production of various lec-tin ligands that might engage various molecules and cell types[173]. Even the glycosyltransferases that participate in selectin-ligand synthesis have yet to be fully identified, and the regulatory events that control the formation of selectin ligands are incompletely understood. In addition, the mechanisms by which selectin ligands differentially affect the immune response, including their ability to modulate intracellular signalling, are just beginning to be studied.

Glycosylation in mammals constructs a glycome that can be distinguished phylogenetically and immunologically from those of lower and divergent organisms, and encompasses glycan linkages with profound immunoregulatory functions. Improved experimental techniques that can rapidly define glycan structure and can image distinct cellular glycoprotein interactions are increasingly relevant. Antibodies that specifically bind the synthetic and degradative enzymes of glycosylation are for the most part not available today, which makes it difficult to detect the molecular interactions that constitute the regulatory machinery of cellular glyco-sylation. Determining the elements of the mammalian glycome and the mechanisms by which the glycome is constructed and regulated will provide integrative and profound immunological insights into the biological roles of mammalian glycosylation.

Acknowledgments

Glossary

Glycome

> The biological repertoire of glycan structures

Experimental autoimmune encephalomyelitis

> An experimental model of multiple sclerosis. Autoimmune disease is induced in animals by immunization with myelin or peptides derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord

Immunoreceptor tyrosine-based inhibitory motif

> (ITIM). A short amino-acid sequence (the consensus sequence of which is V/IxYxxV/L, in which x denotes any amino acid) that is found in the cytoplasmic tail of inhibitory receptors. ITIMs are thought to mediate inhibitory signalling by recruiting phosphatases such as sHP1 (sRC-homology-2-domain-containing protein tyrosine phosphatase 1)

Clathrin-enriched microdomain

> A region of the plasma membrane that is associated with clathrin-dependent endocytic mechanisms of protein trafficking and turnover

IgA nephropathy

> An inflammatory disease of the kidney that is associated with the glomerular deposition of IgA

Old-world primates

> A grouping of primates, which includes humans, that are native to Africa and Asia

Henoch–Schönlein purpura

An inflammatory disease that involves the blood vessels and causes vasculitis

Hodgkin's lymphoma

A type of lymphoma that is characterized by the presence of Reed–sternberg cells (derived from B cells) and that spreads between the lymph nodes

References

1. Paulson JC, Blixt O, Collins BE. Sweet spots in functional glycomics. Nature Chem Biol 2006;2:238–248. [PubMed: 16619023]This publication provides an introduction to the definition and concept of the glycome and describes new approaches to research in this field.

2. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. Cell 2006;126:855–867. [PubMed: 16959566]This article reviews the process of glycosylation and the mechanisms by which glycans contribute to molecular interactions that govern health and disease.

3. Raman R, Raguram S, Venkataraman G, Paulson JC, Sasisekharan R. Glycomics: an integrated systems approach to structure–function relationships of glycans. Nature Methods 2005;2:817–824. [PubMed: 16278650]

4. Haltiwanger RS, Lowe JB. Role of glycosylation in development. Annu Rev Biochem 2004;73:491–537. [PubMed: 15189151]This article reviews the developmental biology of glycosylation and the mechanisms by which glycans control ontogeny.

5. Caramelo JJ, Parodi AJ. How sugars convey information on protein conformation in the endoplasmic reticulum. Semin Cell Dev Biol 2007;18:732–742. [PubMed: 17997334]

6. Varki A. Nothing in glycobiology makes sense, except in the light of evolution. Cell 2006;126:841–845. [PubMed: 16959563]This review provides a perspective on how glycan function arose and continues to change in the evolution of organisms.

7. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. Annu Rev Biochem 2004;73:1019–1049. [PubMed: 15189166]

8. Lowe JB, Marth JD. A genetic approach to mammalian glycan function. Annu Rev Biochem 2003;72:643–691. [PubMed: 12676797]

9. Comelli EM, et al. A focused microarray approach to functional glycomics: transcriptional regulation of the glycome. Glycobiology 2005;16:117–131. [PubMed: 16237199]

10. Wu X, et al. Mutation of the COG complex subunit COG7 causes a lethal congenital disorder. Nature Med 2004;10:518–523. [PubMed: 15107842]

11. Hart GW. Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins. Annu Rev Biochem 1997;66:315–335. [PubMed: 9242909]

12. Dias WB, Hart GW. O-GlcNAc modification in diabetes and Alzheimer's disease. Mol Biosyst 2007;3:766–772. [PubMed: 17940659]

13. Sharon N. Lectins: carbohydrate-specific reagents and biological recognition molecules. J Biol Chem 2007;282:2753–2764. [PubMed: 17145746]This publication describes the origins and activities of lectins as proteinaceous receptors that bind glycans.

14. Taylor ME, Drickamer K. Paradigms for glycan-binding receptors in cell adhesion. Curr Opin Cell Biol 2007;19:572–577. [PubMed: 17942297]This article describes the various lectins and their binding activities in adhesive functions between cells.

15. Goldstein IJ. Lectin structure-activity: the story is never over. J Agric Food Chem 2002;50:6583–6585. [PubMed: 12381155]This publication describes the extent of origins and activities of lectins.

16. Gagneux P, Varki A. Evolutionary considerations in relating oligosaccharide diversity to biological function. Glycobiology 1999;9:747–755. [PubMed: 10406840]

17. Medzhitov R, Janeway JC. The Toll receptor family and microbial recognition. Trends Microbiol 2000;8:452–456. [PubMed: 11044679]

18. van Kooyk Y, Rabinovich GA. Protein–glycan interactions in the control of innate and adaptive immune responses. Nature Immunol 2008;9:593–601. [PubMed: 18490910]

19. Baum LG. Developing a taste for sweets. Immunity 2002;16:5–8. [PubMed: 11825561]

20. Lowe JB. Glycosylation, immunity, and autoimmunity. Cell 2001;104:809–812. [PubMed: 11290318]

21. Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. Glycosylation and the immune system. Science 2001;291:2370–2374. [PubMed: 11269318]

22. Lowe JB. Glycosyltransferases and glycan structures contributing to the adhesive activities of L-, E- and P-selectin counter-receptors. Biochem Soc Symp 2002;69:33–45. [PubMed: 12655772]

23. Rosen SD. Ligands for L-selectin: homing, inflammation, and beyond. Annu Rev Immunol 2004;22:129–156. [PubMed: 15032576]

24. McEver RP. Leukocyte interactions mediated by selectins. Thromb Haemost 1991;66:80–87. [PubMed: 1718051]

25. Lasky LA. Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 1992;258:964–969. [PubMed: 1439808]

26. Ley K, Kansas GS. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. Nature Rev Immunol 2004;4:325–335. [PubMed: 15122198]

27. Rossi FMV, et al. Recruitment of adult thymic progenitors is regulated by P-selectin and its ligand PSGL-1. Nature Immunol 2005;6:626–634. [PubMed: 15880112]

28. McEver R. Selectin–carbohydrate interactions during inflammation and metastasis. Glycoconj J 1997;14:585–591. [PubMed: 9298691]

29. McEver RP, Cummings RD. Role of PSGL-1 binding to selectins in leukocyte recruitment. J Clin Invest 1997;100:S97–103. [PubMed: 9413410]

30. Hidalgo A, Peired AJ, Wild MK, Vestweber D, Frenette PS. Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. Immunity 2007;26:477–489. [PubMed: 17442598]

31. Zisoulis DG, Kansas GS. H-Ras and phosphoinositide 3-kinase co-operate to induce $\alpha(1,3)$-fucosyltransferase VII expression in Jurkat T cells. J Biol Chem 2004;279:39495–39504. [PubMed: 15262995]

32. Underhill GH, et al. A crucial role for T-bet in selectin ligand expression in T helper 1 (Th1) cells. Blood 2005;106:3867–3873. [PubMed: 16099875]

33. Kang R, et al. Transcriptional regulation of the *N*-acetylglucosaminyltransferase V gene in human bile duct carcinoma cells (HuCC-T1) is mediated by Ets-1. J Biol Chem 1996;271:26706–26712. [PubMed: 8900148]

34. Maly P, et al. The $\alpha(1, 3)$fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. Cell 1996;86:643–653. [PubMed: 8752218]

35. Homeister JW, et al. The $\alpha(1, 3)$fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. Immunity 2001;15:115–126. [PubMed: 11485743]

36. Smith PL, et al. Conditional control of selectin ligand expression and global fucosylation events in mice with a targeted mutation at the FX locus. J Cell Biol 2002;158:801–815. [PubMed: 12186857]

37. Ellies LG, et al. Sialyltransferase specificity in selectin ligand formation. Blood 2002;100:3618–3425. [PubMed: 12393657]

38. Sperandio M, et al. α2,3-sialyltransferase-IV is essential for L-selectin ligand function in inflammation. Eur J Immunol 2006;36:3207–3215. [PubMed: 17111351]

39. Frommhold, et al. Sialyltransferase ST3Gal-IV controls CXCR2-mediated firm leukocyte arrest during inflammation. J Exp Med 2008;205:1435–1446. [PubMed: 18519646]

40. Ellies LG, et al. Core 2 oligosaccharide biosynthesis distinguishes between selectin ligands essential for leukocyte homing and inflammation. Immunity 1998;9:881–890. [PubMed: 9881978]

41. Sperandio M, et al. Differential requirements for core2 glucosaminyltransferase for endothelial L-selectin ligand function *in vivo*. J Immunol 2001;16:7, 2268–2274.

42. Gauguet JM, Rosen SD, Marth JD, von Andrian UH. Core 2 branching β1,6-*N*-acetyl-glucosaminyltransferase and high endothelial cell *N*-acetylglucosamine-6-sulfotransferase exert differential control over B- and T-lymphocyte homing to peripheral lymph nodes. Blood 2004;104:4104–4112. [PubMed: 15319280]

43. Yeh JC, et al. Novel sulfated lymphocyte homing receptors and their control by a core1 extension β1, 3-*N*-acetylglucosaminyltransferase. Cell 2001;105:957–969. [PubMed: 11439191]

44. Mitoma J, et al. Critical functions of N-glycans in L-selectin-mediated lymphocyte homing and recruitment. Nature Immunol 2007;8:409–418. [PubMed: 17334369]

45. Kawashima H, et al. *N*-acetylglucosamine-6-*O*-sulfotransferases 1 and 2 cooperatively control lymphocyte homing through L-selectin ligand biosynthesis in high endothelial venules. Nature Immunol 2005;6:1096–1164. [PubMed: 16227985]

46. Uchimura K, et al. A major class of L-selectin ligands is eliminated in mice deficient in two sulfotransferases expressed in high endothelial venules. Nature Immunol 2005;6:1105–1113. [PubMed: 16227986]

47. Hidari KI, Weyrich AS, Zimmerman GA, McEver RP. Engagement of P-selectin glycoprotein ligand-1 enhances tyrosine phosphorylation and activates mitogen-activated protein kinases in human neutrophils. J Biol Chem 1997;272:28750–28756. [PubMed: 9353345]

48. Simon SI, Hu Y, Vestweber D, Smith CW. Neutrophil tethering on E-selectin activates β2 integrin binding to ICAM-1 through a mitogen-activated protein kinase signal transduction pathway. J Immunol 2000;164:4348–4358. [PubMed: 10754335]

49. Urzainqui A, et al. ITAM-based interaction of ERM proteins with Syk mediates signaling by the leukocyte adhesion receptor PSGL-1. Immunity 2002;17:401–412. [PubMed: 12387735]

50. Zarbock A, Lowell CA, Ley K. Spleen tyrosine kinase Syk is necessary for E-selectin-induced $\alpha_L\beta_2$ integrin-mediated rolling on intercellular adhesion molecule-1. Immunity 2007;26:773–783. [PubMed: 17543554]This study links selectin binding to signal transduction by tyrosine kinases.

51. Kim YJ, Borsig L, Varki NM, Varki A. P-selectin deficiency attenuates tumor growth and metastasis. Proc Natl Acad Sci USA 1998;95:9325–9380. [PubMed: 9689079]

52. Chen S, Fukuda M. Cell type-specific roles of carbohydrates in tumor metastasis. Methods Enzymol 2006;416:371–380. [PubMed: 17113879]

53. Romano SJ. Selectin antagonists: therapeutic potential in asthma and COPD. Treat Respir Med 2005;4:85–94. [PubMed: 15813660]

54. Uchimura K, Rosen SD. Sulfated L-selectin ligands as a therapeutic target in chronic inflammation. Trends Immunol 2006;27:559–565. [PubMed: 17049924]

55. Woollard KJ, Chin-Dusting J. Therapeutic targeting of P-selectin in atherosclerosis. Inflamm Allergy Drug Targets 2007;6:69–74. [PubMed: 17352691]

56. Sackstein R, et al. *Ex vivo* glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. Nature Med 2008;14:181–187. [PubMed: 18193058]

57. Rapoport EM, Kurmyshkina OV, Bovin NV. Mammalian galectins: structure, carbohydrate specificity, and functions. Biochemistry (Mosc) 2008;73:393–405. [PubMed: 18457568]

58. Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by *Mgat5* N-glycosylation. Nature 2001;409:733–739. [PubMed: 11217864]

59. Lee SU, et al. N-glycan processing deficiency promotes spontaneous inflammatory demyelination and neurodegeneration. J Biol Chem 2007;282:33725–33734. [PubMed: 17855338]

60. Demotte N, et al. Restoring the association of the T cell receptor with CD8 reverses anergy in human tumor-infiltrating lymphocytes. Immunity 2008;28:414–424. [PubMed: 18342010]

61. Liu SD, et al. Endogenous galectin-1 enforces class I-restricted TCR functional fate decisions in thymocytes. Blood 2008;112:120–130. [PubMed: 18323414]

62. Grigorian A, et al. Control of T cell-mediated autoimmunity by metabolite flux to N-glycan biosynthesis. J Biol Chem 2007;282:20027–20035. [PubMed: 17488719]

63. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nature Rev Immunol 2007;7:255–266. [PubMed: 17380156]This review covers the structure and activities of the Siglec family of lectins and their immunological functions.

64. Tedder TF, Tuscano J, Sato S, Kehrl JH. CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. Annu Rev Immunol 1997;15:481–504. [PubMed: 9143697]

65. Hennet T, Chui D, Paulson JC, Marth JD. Immune regulation by the ST6Gal sialyltransferase. Proc Natl Acad Sci USA 1998;95:4504–4509. [PubMed: 9539767]

66. Han S, Collins BE, Bengtson P, Paulson JC. Homo-multimeric complexes of CD22 in B cells revealed by protein–glycan cross-linking. Nature Chem Biol 2005;1:93–97. [PubMed: 16408005]

67. Collins BE, Smith BA, Bengtson P, Paulson JC. Ablation of CD22 in ligand-deficient mice restores B cell receptor signaling. Nature Immunol 2006;7:199–206. [PubMed: 16369536]

68. Grewal PK, et al. ST6Gal-I restrains CD22-dependent antigen receptor endocytosis and Shp-1 recruitment in normal and pathogenic immune signaling. Mol Cell Biol 2006;26:4970–4981. [PubMed: 16782884]

69. Santos L, et al. Dendritic cell-dependent inhibition of B cell proliferation requires CD22. J Immunol 2008;180:4561–4569. [PubMed: 18354178]

70. Kobata A. The N-linked sugar chains of human immunoglobulin G: their unique pattern, and their functional roles. Biochim Biophys Acta 2008;1780:472–478. [PubMed: 17659840]

71. Wright A, Morrison SL. Effect of glycosylation on antibody function: implications for genetic engineering. Trends Biotech 1997;15:26–32.

72. Parekh RB, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 1985;316:452–457. [PubMed: 3927174]

73. Hoffmeister KM, et al. Glycosylation restores survival of chilled blood platelets. Science 2003;301:1531–1534. [PubMed: 12970565]

74. Jones AJS, et al. Selective clearance of glycoforms of a complex glycoprotein pharmaceutical caused by terminal *N*-acetylglucosamine is similar in humans and cynomolgus monkeys. Glycobiology 2007;17:529–540. [PubMed: 17331977]

75. Hiki Y, et al. Mass spectrometry proves under-O-glycosylation of glomerular IgA1 in IgA nephropathy. Kidney Int 2001;59:1077–1085. [PubMed: 11231363]

76. Nishie T, et al. Development of immunoglobulin A nephropathy-like disease in β-1,4-galactosyl-transferase-I-deficient mice. Am J Pathol 2007;170:447–456. [PubMed: 17255313]

77. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 2006;313:670–673. [PubMed: 16888140]

78. Anthony RM, et al. Racapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 2008;320:373–376. [PubMed: 18420934]

79. Wang X, et al. Dysregulation of TGF-β1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. Proc Natl Acad Sci USA 2005;102:15791–15794. [PubMed: 16236725]

80. Wang X, et al. Phenotype changes of fut8 knockout mouse: core fucosylation is crucial for the function of growth factor receptor(s). Methods Enzymol 2006;417:11–22. [PubMed: 17132494]

81. Vogt G, et al. Gains of glycosylation comprise an unexpectedly large group of pathogenic mutations. Nature Genet 2005;37:692–700. [PubMed: 15924140]

82. Macher BA, Galili U. The Galα1, 3Galβ1, 4GlcNAc-R. (α-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. Biochim Biophys Acta 2007;1780:75–88. [PubMed: 18047841]

82. Padler-Karavani V, et al. Diversity in specificity, abundance and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease. Glycobiology. Jul 31;2008 10.1093/glycob/cwn072

84. Ju T, Cummings RD. Protein glycosylation: chaperone mutation in Tn syndrome. Nature 2005;437:1252. [PubMed: 16251947]

85. Green RS, et al. Mammalian N-glycan branching protects against innate immune self-recognition and inflammation in autoimmune disease pathogenesis. Immunity 2007;27:308–320. [PubMed: 17681821]

86. Bianco GA, Toscano MA, Ilarregui JM, Rabinovich GA. Impact of protein–glycan interactions in the regulation of autoimmunity and chronic inflammation. Autoimmunity Rev 2006;5:349–356. [PubMed: 16782561]

87. Baum LG, et al. Amelioration of graft versus host disease by galectin-1. Clin Immunol 2003;109:295–307. [PubMed: 14697744]

88. Rubinstein N, et al. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: a potential mechanism of tumor-immune privilege. Cancer Cell 2004;5:241–251. [PubMed: 15050916]

89. Perone MJ, et al. Dendritic cells expressing transgenic galectin-1 delay onset of autoimmune diabetes in mice. J Immunol 2006;177:5278–5289. [PubMed: 17015713]

90. Seki M, et al. Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin Immunol 2008;127:78–88. [PubMed: 18282810]

91. Barrionuevo P, et al. A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. J Immunol 2007;178:436–445. [PubMed: 17182582]

92. Blois SM, et al. A pivotal role for galectin-1 in fetomaternal tolerance. Nature Med 2007;13:1450–1457. [PubMed: 18026113]

93. Taylor KR, et al. Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44 and MD-2. J Biol Chem 2007;282:18265–18275. [PubMed: 17400552]

94. Jiang D, Liang J, Noble PW. Hyaluronan in tissue injury and repair. Annu Rev Cell Develop Biol 2007;23:435–461.

95. Girish KS, Kemparaju K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. Life Sci 2007;80:1921–1943. [PubMed: 17408700]

96. Forsberg E, et al. Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. Nature 1999;400:773–776. [PubMed: 10466727]

97. Humphries DE, et al. Heparin is essential for the storage of specific granule proteases in mast cells. Nature 1999;400:769–772. [PubMed: 10466726]

98. Carbone FR, Gleeson PA. Carbohydrates and antigen recognition by T cells. Glycobiology 1997;7:725–730. [PubMed: 9376674]

99. Zhao XJ, Cheung NK. GD2 oligosaccharide: target for cytotoxic T lymphocytes. J Exp Med 1995;182:67–74. [PubMed: 7540657]

100. Liu SD, et al. Endogenous galectin-1 enforces class I-restricted TCR functional fate decisions in thymocytes. Blood 2008;112:120–130. [PubMed: 18323414]

101. Corthay A, et al. Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis. Eur J Immunol 1998;28:2580–2590. [PubMed: 9710235]

102. Wells L, Vosseller K, Hart GW. Glycosylation of nucleocytoplasmic proteins: signal transduction and *O*-GlcNAc. Science 2001;291:2376–2378. [PubMed: 11269319]

103. Zajonc DM, Kronenberg M. CD1 mediated T cell recognition of glycolipids. Curr Opin Struct Biol 2007;17:521–529. [PubMed: 17951048]

104. Daniels MA, et al. CD8 binding to MHC class I molecules is influenced by T cell maturation and glycosylation. Immunity 2001;15:1051–1061. [PubMed: 11754824]

105. Moody AM, et al. Developmentally regulated glycosylation of the CD8αβ coreceptor stalk modulates ligand binding. Cell 2001;107:501–512. [PubMed: 11719190]

106. Kao C, Daniels MA, Jameson SC. Loss of CD8 and TCR binding to class I MHC ligands following T cell activation. Int Immunol 2005;17:1607–1617. [PubMed: 16263755]

107. Moody AM, et al. Sialic acid capping of CD8β core 1-O-glycans controls thymocyte-major histocompatibility complex class I interaction. J Biol Chem 2003;278:7240–7246. [PubMed: 12459555]

108. Kao C, Sandau MM, Daniels MA, Jameson SC. The sialyltransferase ST3Gal-I is not required for regulation of CD8-class I MHC binding during T cell development. J Immunol 2006;176:7421–7430. [PubMed: 16751387]

109. Van Dyken SJ, Green RS, Marth JD. Structural and mechanistic features of protein O glycosylation linked to CD8^{+} T-cell apoptosis. Mol Cell Biol 2007;27:1096–1111. [PubMed: 17101770]

110. Zhou L, et al. Notch-dependent control of myelopoiesis is regulated by fucosylation. Blood 2008;112:308–319. [PubMed: 18359890]

111. Irvine KD, Wieschaus E. Fringe, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development. Cell 1994;79:595–606. [PubMed: 7954826]

112. Wu JY, Wen L, Zhang WJ, Rao Y. The secreted product of *Xenopus* gene lunatic fringe, a vertebrate signaling molecule. Science 1996;273:355–358. [PubMed: 8662522]

113. Goode S, Perrimon N. Brainiac and fringe are similar pioneer proteins that impart specificity to notch signaling during *Drosophila* development. Cold Spring Harb Symp Quant Biol 1997;62:177–184. [PubMed: 9598350]

114. Johnston SH, et al. A family of mammalian Fringe genes implicated in boundary determination and the Notch pathway. Development 1997;124:2245–2254. [PubMed: 9187150]

115. Panin VM, Papayannopoulos V, Wilson R, Irvine KD. Fringe modulates Notch–ligand interactions. Nature 1997;387:908–912. [PubMed: 9202123]

116. Brückner K, Perez L, Clausen H, Cohen S. Glycosyltransferase activity of Fringe modulates Notch–Delta interactions. Nature 2000;406:411–415. [PubMed: 10935637]

117. Moloney DJ, et al. Fringe is a glycosyltransferase that modifies Notch. Nature 2000;406:369–375. [PubMed: 10935626]

118. Chen J, Moloney DJ, Stanley P. Fringe modulation of Jagged1-induced Notch signaling requires the action of β4galactosyltransferase-1. Proc Natl Acad Sci USA 2001;98:13716–13721. [PubMed: 11707585]

119. Wang Y, et al. Modification of epidermal growth factor-like repeats with *O*-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase. J Biol Chem 2001;276:40338–40345. [PubMed: 11524432]

120. Okajima T, Irvine KD. Regulation of Notch signaling by O-linked fucose. Cell 2002;111:893–904. [PubMed: 12526814]

121. Okajima T, Xu A, Irvine KD. Modulation of Notch-ligand binding by protein *O*-fucosyltransferase 1 and Fringe. J Biol Chem 2003;278:42340–42345. [PubMed: 12909620]

122. Shi S, Stanley P. Protein *O*-fucosyltransferase 1 is an essential component of Notch signaling pathways. Proc Natl Acad Sci USA 2003;100:5234–5239. [PubMed: 12697902]

123. Yang LT, et al. Fringe glycosyltransferases differentially modulate Notch1 proteolysis induced by Delta1 and Jagged1. Mol Biol Cell 2005;16:927–942. [PubMed: 15574878]

124. Okajima T, Xu A, Lei L, Irvine KD. Chaperone activity of protein *O*-fucosyltransferase 1 promotes Notch receptor folding. Science 2005;307:1599–1603. [PubMed: 15692013]

125. Acar M, et al. Rumi is a CAP10 domain glycosyltransferase that modifies Notch and is required for Notch signaling. Cell 2008;132:247–258. [PubMed: 18243100]

126. Stanley P. Glucose: a novel regulator of Notch signaling. ACS Chem Biol 2008;3:210–213. [PubMed: 18422303]

127. Robey E, et al. An activated form of Notch influences the choice between CD4 and CD8 T cell lineages. Cell 1996;87:483–492. [PubMed: 8898201]

128. Koch U, et al. Subversion of the T/B lineage decision in the thymus by lunatic fringe-mediated inhibition of Notch-1. Immunity 2001;15:225–234. [PubMed: 11520458]

129. Visan I, et al. Regulation of T lymphopoiesis by Notch1 and Lunatic fringe-mediated competition for intrathymic niches. Nature Immunol 2006;7:634–643. [PubMed: 16699526]

130. Jenkinson EJ, Jenkinson WE, Rossi SW, Anderson G. The thymus and T-cell commitment: the right niche for Notch. Nature Rev Immunol 2006;6:551–555. [PubMed: 16799474]

131. Tanagaki K, et al. Notch–RBP-J signaling in involved in cell fate determination of marginal zone B cells. Nature Immunol 2002;3:443–450. [PubMed: 11967543]

132. Hozumi K, et al. Delta-like 1 is necessary for the generation of marginal zone B cells but not T cells *in vivo*. Nature Immunol 2004;5:638–644. [PubMed: 15146182]

133. Santos MA, et al. Notch1 engagement by Delta-like-1 promotes differentiation of B lymphocytes to antibody-secreting cells. Proc Natl Acad Sci USA 2007;104:15454–15459. [PubMed: 17878313]

134. Cheng P, Gabrilovich D. Notch signaling in differentiation and function of dendritic cells. Immunol Res 2008;41:1–14. [PubMed: 18008056]

135. Kijima, et al. Dendritic cell-mediated NK cell activation is controlled by Jagged2–Notch interaction. Proc Natl Acad Sci USA 2008;105:7010–7015. [PubMed: 18458347]

136. Okamoto, et al. Essential role of Notch signaling in effector memory CD8^{+} T cell-mediated airway hyperresponsiveness and inflammation. J Exp Med 2008;205:1087–1097. [PubMed: 18426985]

137. Schaller MA, et al. Notch ligand Delta-like 4 regulates disease pathogenesis during respiratory viral infections by modulating Th2 cytokines. J Exp Med 2007;204:2925–2934. [PubMed: 17998388]

138. Sun J, Krawczyk CJ, Pearce EJ. Suppression of Th2 cell development by Notch ligands Delta1 and Delta4. J Immunol 2008;180:1655–1661. [PubMed: 18209061]

139. Morgan R, et al. *N*-acetylglucosaminyltransferase V (Mgat5)-mediated N-glycosylation negatively regulates Th1 cytokine production by T cells. J Immunol 2004;173:7200–7208. [PubMed: 15585841]

140. Juszczynski P, et al. The AP1-dependent secretion of galectin-1 by Reed–Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. Proc Natl Acad Sci USA 2007;104:13134–13139. [PubMed: 17670934]

141. Toscano MA, et al. Differential glycosylation of T_H1, T_H2, and T_H-17 effector cells selectively regulates susceptibility to cell death. Nature Immunol 2007;8:825–834. [PubMed: 17589510]

142. Thomas PG, Harn DA. Immune biasing by helminth glycans. Cell Microbiol 2004;6:13–22. [PubMed: 14678327]

143. Opferman JT, Korsmeyer SJ. Apoptosis in the development and maintenance of the immune system. Nature Immunol 2003;4:410–415. [PubMed: 12719730]

144. Hernandez JD, Baum LG. Ah, sweet mystery of death! Galectins and control of cell fate. Glycobiology 2002;12:127R–136R.This publication reviews galectins in immune-cell apoptosis.

145. Hsu DK, Yang RY, Liu FT. Galectins in apoptosis. Methods Enzymol 2006;417:256–273. [PubMed: 17132510]

146. Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. Nature 1995;378:736–739. [PubMed: 7501023]

147. Stowell SR, et al. Human galectin-1, -2, and -4 induce surface expression of phosphatidylserine in activated human neutrophils but not in activated T cells. Blood 2008;109:219–227. [PubMed: 16940423]

148. Stowell SR, et al. Differential roles of galectin-1 and galectin-3 in regulating leukcoyte viability and cytokine secretion. J Immunol 2008;180:3091–3102. [PubMed: 18292532]

149. Stowell SR, et al. Dimeric galectin-8 induces phosphatidylserine exposure in leukocytes through polylactosamine recognition by the carboxyl terminal domain. J Biol Chem 2008;283:20547–20559. [PubMed: 18456665]

150. Dias-Baruffi M, et al. Dimeric galectin-1 induces surface exposure of phosphatidylserine and phagocytic recognition of leukocytes without inducing apoptosis. J Biol Chem 2003;278:41282–41293. [PubMed: 12853445]

151. Vespa GN, et al. Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibits IL-2 production and proliferation. J Immunol 1999;162:799–806. [PubMed: 9916701]

152. Pace KE, Hahn HP, Pang M, Nguyen JT, Baum LG. CD7 delivers a pro-apoptotic signal during galectin-1-induced T cell death. J Immunol 2000;165:2331–2334. [PubMed: 10946254]

153. Waizel H, Blach M, Hirabayashi J, Kasai KI, Brock J. Involvement of CD2 and CD3 in galectin-1 induced signaling in human Jurkat T-cells. Glycobiology 2000;10:131–140. [PubMed: 10642604]

154. Fuertes MB, et al. Regulated expression of galectin-1 during T-cell activation involves Lck and Fyn kinases and signaling through MEK1/ERK, p38 MAP kinase and p70S6 kinase. Mol Cell Biochem 2004;267:177–185. [PubMed: 15663199]

155. Cabrera PV, et al. Haploinsufficiency of C2GnT-I glycosyltransferase renders T lymphoma cells resistant to cell death. Blood 2006;108:2399–2406. [PubMed: 16778138]

156. Hahn HP, et al. Galectin-1 induces nuclear translocation of endonuclease G in caspase- and cytochrome *c*-independent T cell death. Cell Death Diff 2004;11:1277–1286.

157. Stillman BN, et al. Galectin-2 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. J Immunol 2006;176:778–789. [PubMed: 16393961]

158. Bi S, Earl LA, Jacobs L, Baum LG. Structural features of galectin-9 and galectin-1 that determine distinct T cell death pathways. J Biol Chem 2008;283:12248–12258. [PubMed: 18258591]

159. Priatel JJ, et al. The ST3Gal-I sialyltransferase controls CD8^{+} T cell homeostasis by modulating O-glycan biosynthesis. Immunity 2000;12:273–283. [PubMed: 10755614]

160. Grewal PK, et al. The Ashwell receptor mitigates the lethal coagulopathy of sepsis. Nature Med 2008;14:648–655. [PubMed: 18488037]

161. Brewer CF, Miceli MC, Baum LG. Clusters, bundles, arrays, and lattices: novel mechanisms for lectin-saccharide-mediated cellular interactions. Curr Opin Struct Biol 2002;12:616–623. [PubMed: 12464313]This article reviews the hypothesis that galectins generate a spatially precise, repetitive structure (lattice) that controls glycoprotein trafficking and function on the cell surface.

162. Nieminen J, Kuno A, Hirabayashi J, Sato S. Visualization of galectin-3 oligomerization on the surface of neutrophils and endothelial cells using fluorescence resonance energy transfer. J Biol Chem 2007;282:1374–1383. [PubMed: 17082191]

163. Rabinovich GA, Toscano MA, Jackson SS, Vasta GR. Functions of cell surface galectin–glycoprotein lattices. Curr Opin Struct Biol 2007;17:513–520. [PubMed: 17950594]This publication reviews findings regarding the galectins and their potential to form various lattices that can define functional units and membrane domains.

164. Hernandez JD, et al. Galectin-1 binds different CD43 glycoforms to cluster CD43 and regulate T cell death. J Immunol 2006;177:5328–5336. [PubMed: 17015718]

165. Chen IJ, Chen HL, Demetriou M. Lateral compartmentalization of T cell receptor versus CD45 by galectin-N-glycan binding and microfilaments coordinate basal and activation signaling. J Biol Chem 2007;282:35361–35372. [PubMed: 17897956]

166. Lajoie P, et al. Plasma membrane domain organization regulates EGFR signaling in tumor cells. J Cell Biol 2007;179:341–356. [PubMed: 17938246]

167. Partridge EA, et al. Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. Science 2004;306:120–124. [PubMed: 15459394]

168. Ohtsubo K, Takamatsu S, Minowa MT, Yoshida A, Marth JD. Dietary and genetic control of glucose transporter-2 glycosylation promotes insulin secretion in suppressing diabetes. Cell 2005;123:1307–1321. [PubMed: 16377570]

169. Lau KS, et al. Complex N-glycan number and degree of branching co-operate to regulate cell proliferation and differentiation. Cell 2007;129:123–134. [PubMed: 17418791]

170. van Gisbergen KP, Sanchez-Hernandez M, Geijtenbeek TB, van Kooyk Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. J Exp Med 2005;20:1, 1281–1292.

171. Garcia-Vallejo JJ, et al. DC-SIGN mediates adhesion and rolling of dendritic cells on primary human umbilical vein endothelial cells through Lewis(Y) antigen expressed on ICAM-2. Mol Immunol 2008;45:2359–2369. [PubMed: 18155766]

172. McDonald B, et al. Interaction of CD44 and hyaluronan is the dominant mechanism for neutrophil sequestration in inflamed liver sinusoids. J Exp Med 2008;205:915–927. [PubMed: 18362172]

173. Bax M, et al. Dendritic cell maturation results in pronounced changes in glycan expression affecting recognition by siglecs and galectins. J Immunol 2007;179:8216–8224. [PubMed: 18056365]

174. Hakomori S. Glycosylation defining cancer malignancy: new wine in an old bottle. Proc Natl Acad Sci USA 2002;99:10231–10233. [PubMed: 12149519]

175. Freeze HH. Genetic defects in the human glycome. Nature Rev Genet 2006;7:537–551. [PubMed: 16755287]A review of the human genomic data that are known to contribute to genetic disease by altered glycosylation.

176. Brooks SA, et al. Altered glycosylation of proteins in cancer: what is the potential for new anti-tumour strategies. Anticancer Agents Med Chem 2008;8:2–21. [PubMed: 18220502]

177. Collins BT, et al. Masking of CD22 by cis ligands does not prevent redistribution of CD22 to sites of cell contact. Proc Natl Acad, Sci USA 2004;282:6104–6109. [PubMed: 15079087]
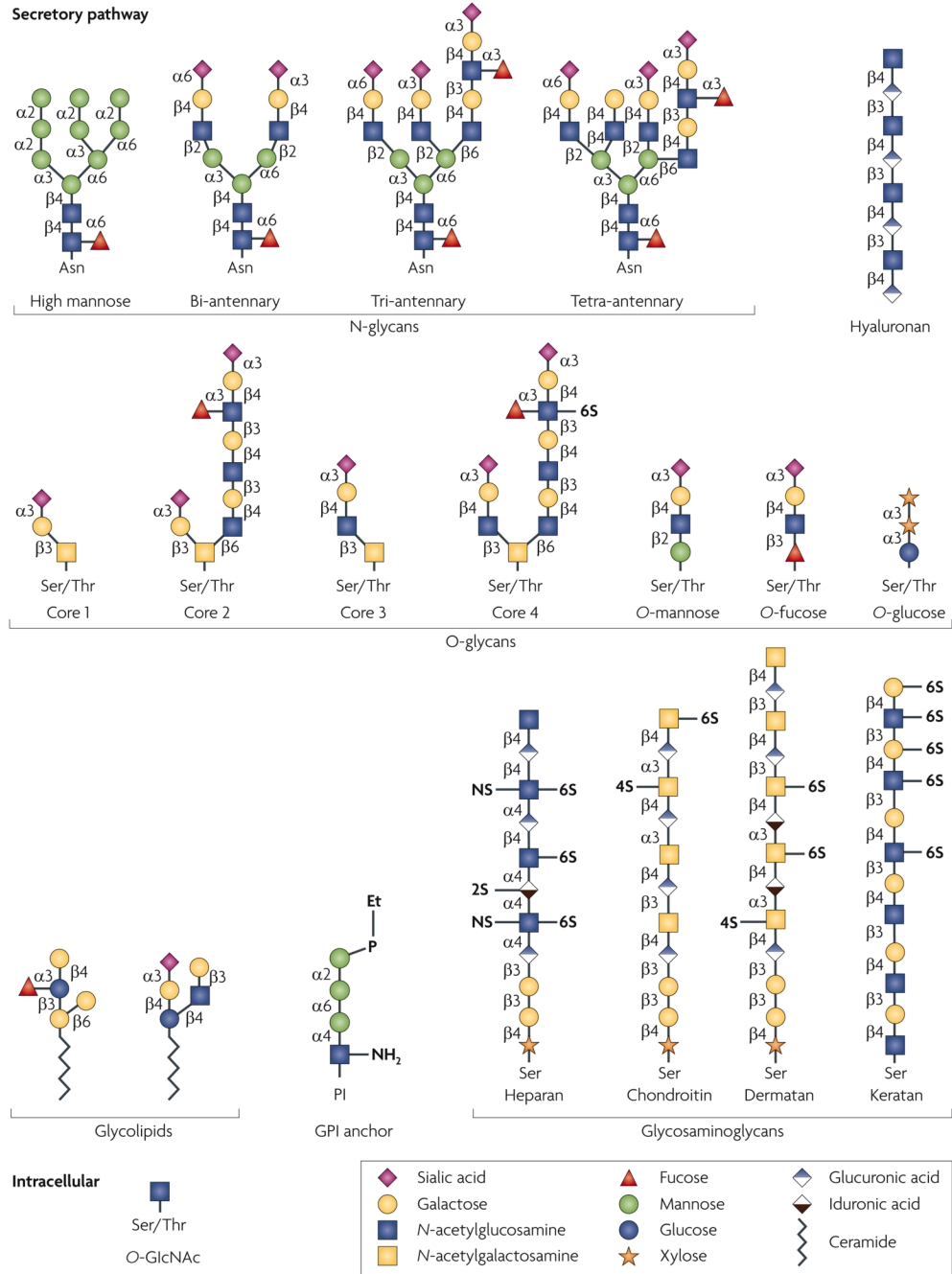
Figure 1. The types of mammalian glycan
Glycan structures of the six classes of secretory glycan (N-glycans, hyaluronan, O-glycans, glycolipids, glycosylphosphatidylinositol (GPI) anchor and glycosaminoglycans) and the single intracellular glycan O-linked β-*N*-acetylglucosamine (*O*-GlcNAc) are shown. Representative examples of each type are indicated using the symbol nomenclature for monosaccharides (see key). Multiple and multi-antennary examples of the predominant mature, complex-type N-glycans are shown, including the high-mannose N-glycan that is found attached to some glycoproteins. Examples of core 1–4 O-glycans are depicted, as well as *O*-mannose, *O*-fucose and *O*-glucose structures. Core 5–7 O-glycans are not shown. The GPI anchor and examples of the glycosaminoglycans and glycolipids are also depicted. In all

examples, glycan linkages are identified by the anomeric configuration (α orβ) of the donor saccharide following its linkage to the ring position (1–6) of the glycan acceptor. Et–P denotes a phosphoethanolamine linkage. NS, 2S, 4S and 6S denote the sulphation positions of the glycosaminoglycan chains. Asn, asparagine; Ser, serine; Thr, threonine.
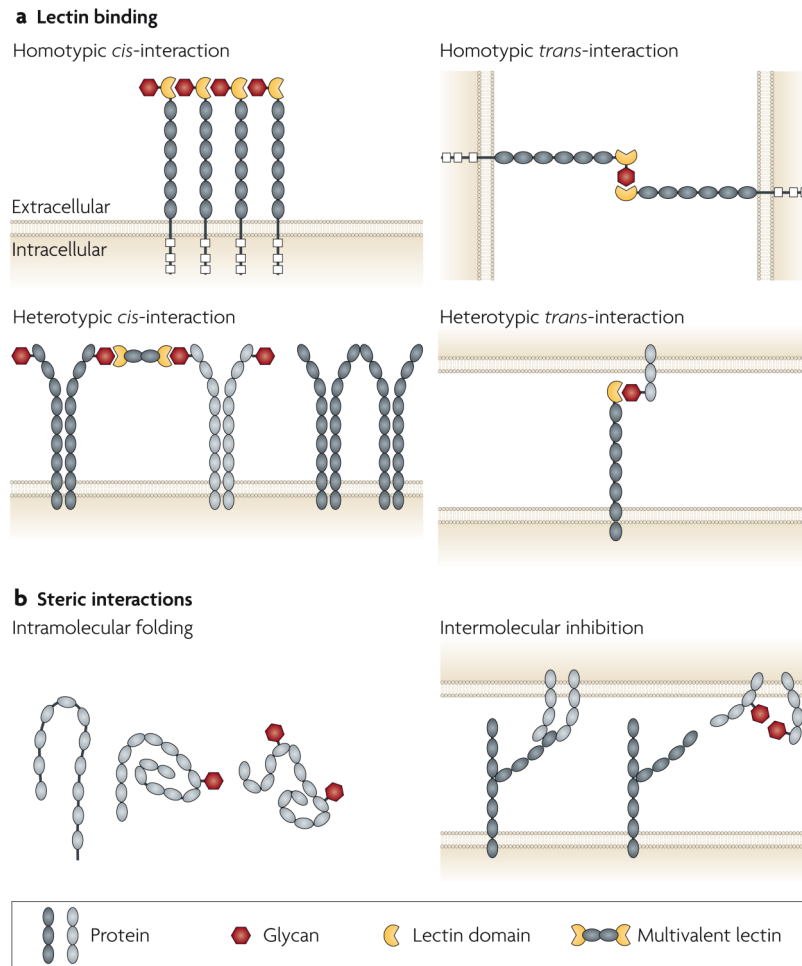
Figure 2. Basic mechanisms of glycan function in the immune system
Glycans can function as ligands for lectins (**a**) and as steric elements that alter molecular interactions at the cell surface and between extracellular compartments (**b**). Interactions can involve the same cell (*cis*) or different cells (*trans*), and can be either homotypic or heterotypic, and intramolecular or intermolecular. It is often difficult to distinguish steric effects of glycans from alterations in lectin–glycan binding. This figure is idealized and not drawn to scale.
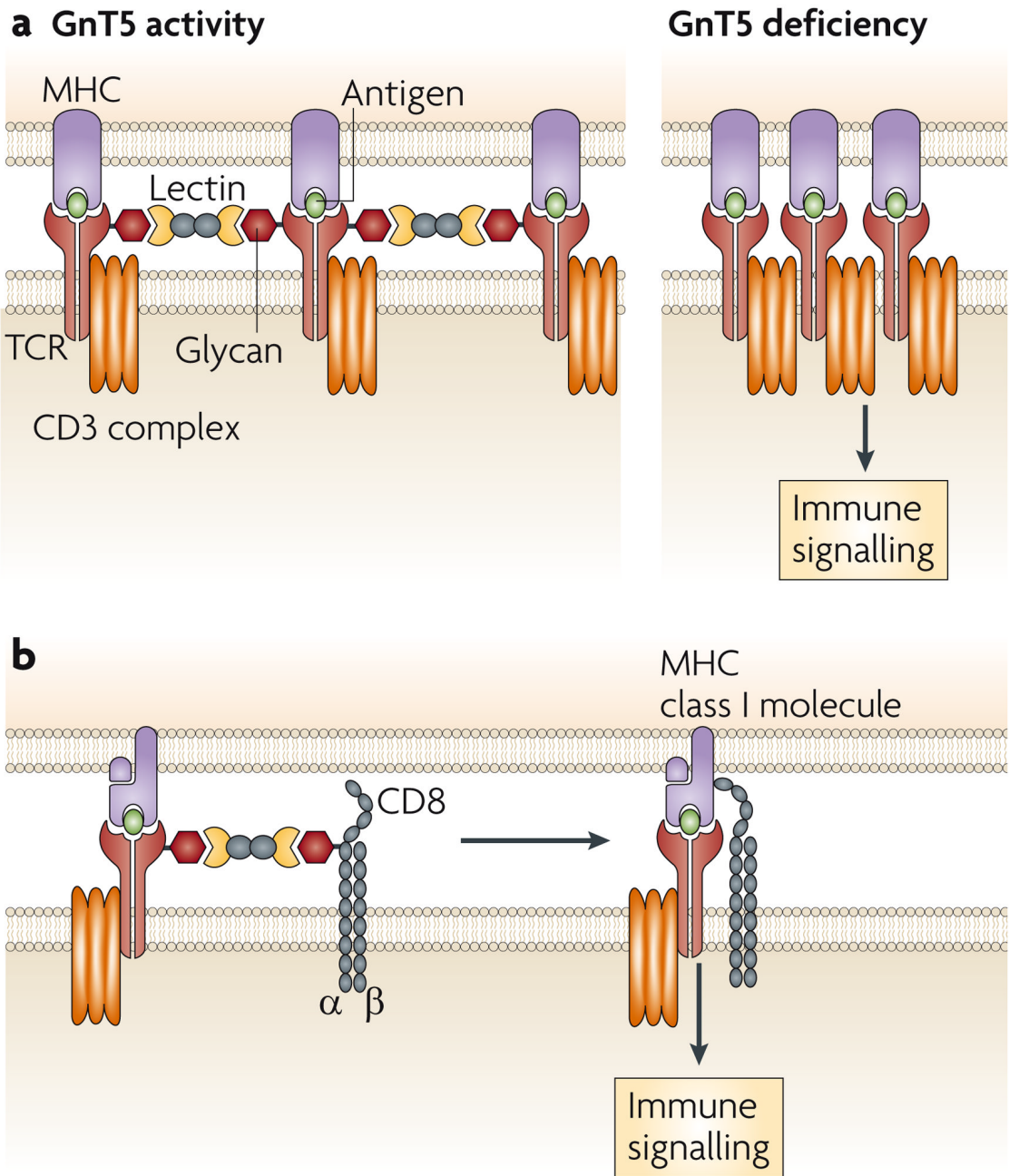
Figure 3. Glycans in T-cell-receptor activation
a. Co-clustering of αβ T-cell receptors (TCRs) decreases in the presence of N-glycan branching on the TCR through lectin binding involving one or more of the galectins. Loss of GnT5 glycosyltransferase activity, which mediates N-glycan branching, and therefore lectin binding, increases the propensity for TCR and co-receptor co-clustering and decreases the threshold of TCR activation. **b**. Lectin binding might also control TCR signalling in cytotoxic T cells by inhibiting the association of the TCR with the CD8 co-receptor.
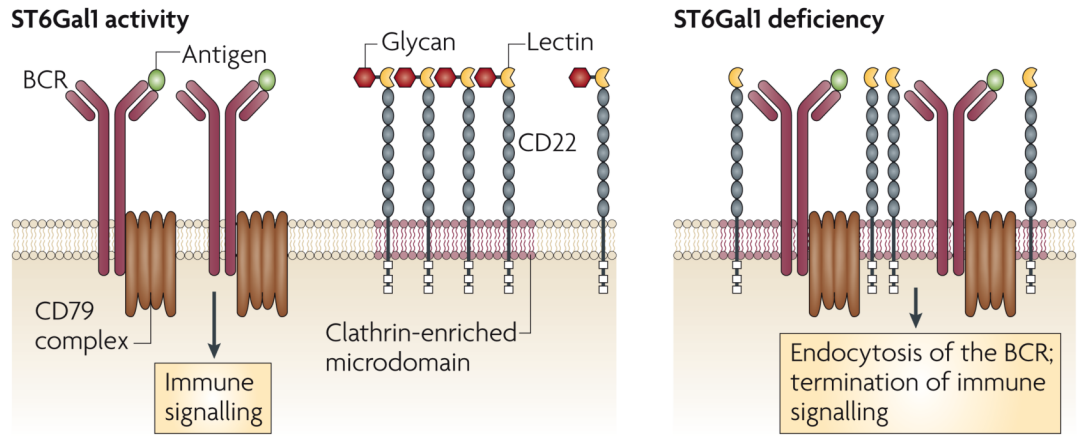
Figure 4. Glycans in B-cell-receptor activation
The threshold of B-cell receptor (BCR) activation is modulated by ST6Gal1 and CD22, a member of the Siglec (sialic-acid-binding immunoglobulin-like lectin) family that has a negative role in BCR signalling. CD22 Siglec binding to its glycan ligand promotes homotypic CD22–CD22 interactions, thereby decreasing the frequency of interaction of CD22 with the IgM BCR on mature naive B cells. Loss of CD22 Siglec ligands owing to ST6Gal1 sialyltransferase deficiency markedly increases the co-localization of CD22 with the BCR, thereby increasing the threshold of immune signalling and promoting endocytosis of the BCR in a CD22-dependent manner.
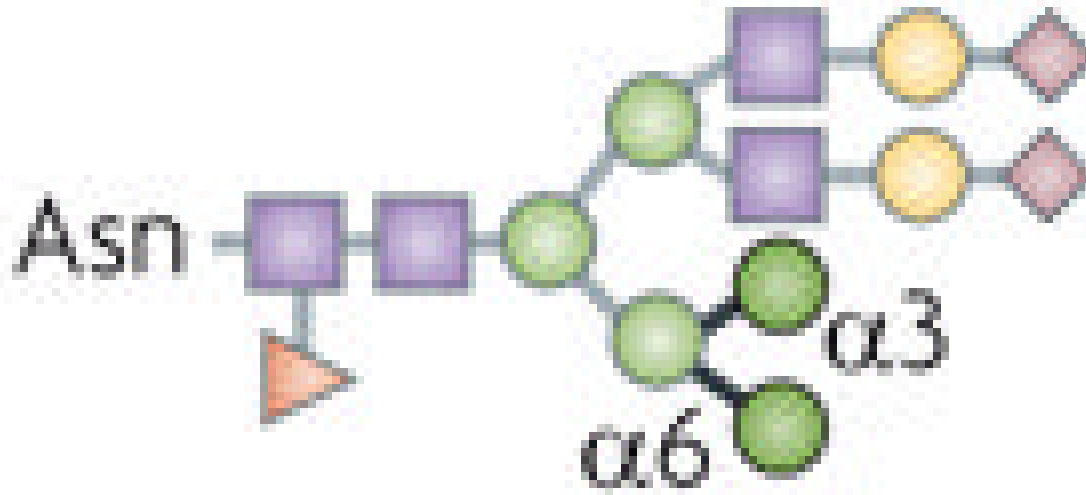
Table 1

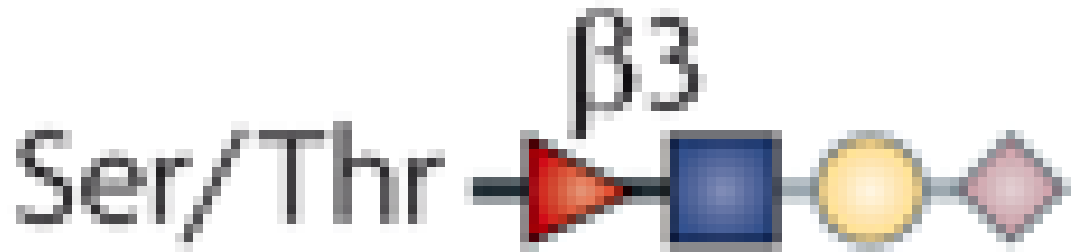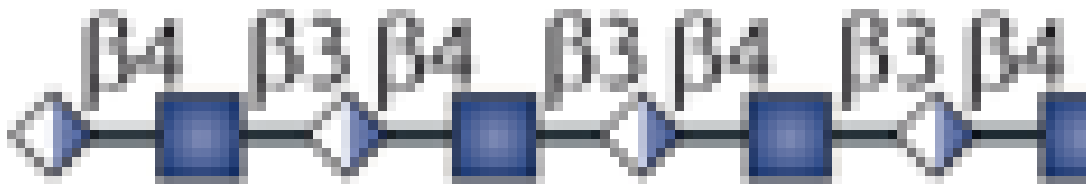Mammalian glycan structures that are linked to immune functions

Glvcan*

sLe^x

6-sulpho-sLe^x

Core 1 O-glycan

Terminal GlcNAc

N-acetyl-lactosamine and polylactosamine

β1,6 N-glycan branch

Sia6LacNAc
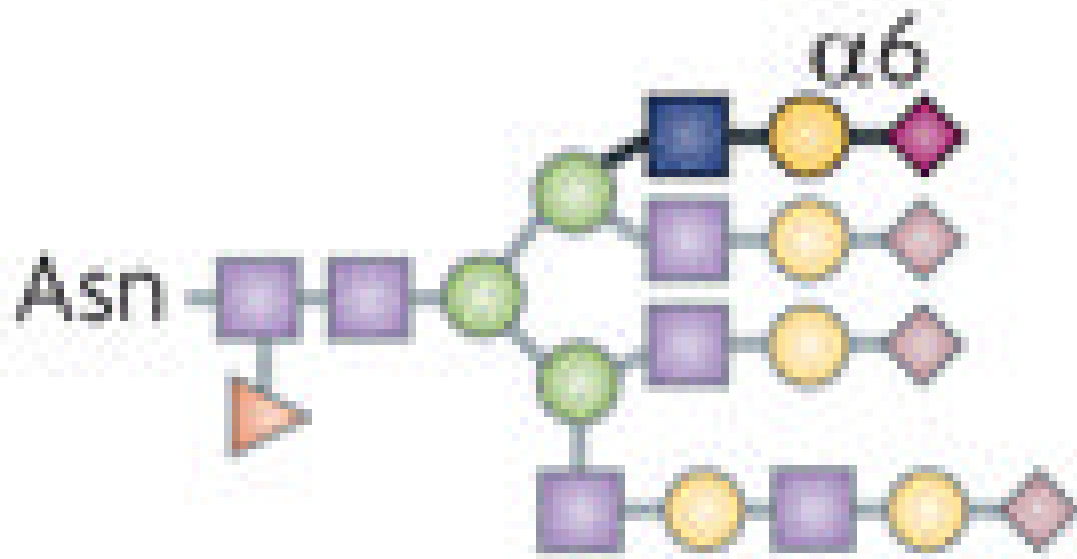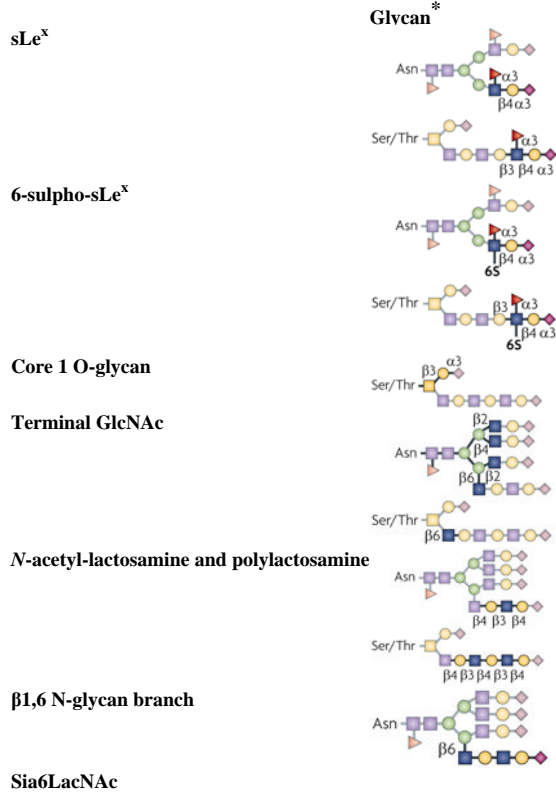
Glycan[*]

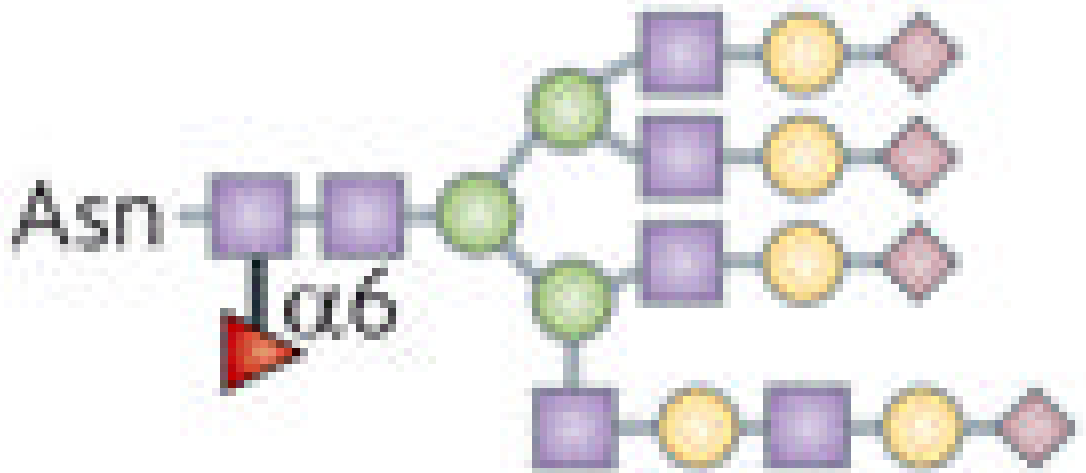**Hybrid N-glycan**

O-**fucose glycan**

O-**glucose glycan**
Hyaluronan

N-glycan

[*] Glycan structure is indicated using the symbol nomenclature for monosaccharides; see FIG. 1 for further information.

[‡]Glycosyltransferase and glycosidase enzymes that are known to be responsible for the presence of the indicated glycan linkages, whether owing to their regulated expression or lack of expression.

[§]Recognition proteins that include classically defined lectins, which function by binding to the highlighted glycan structures. αM-II, α-mannosidase-II; β1,4GalT, β1,4-galactosyltransferase; β1,3GnT, β1,3-glucosaminyltransferase; BCR, B-cell receptor; DC, dendritic cell; Fut, fucosyltransferase; GCNT1, core 2 β1,4-glucosaminyltransferase 1; GlcNAc, β-*N*-acetylglucosamine; GlcNAc6ST, glycan sulphotransferase; GnT, *N*-acetylglucosaminyltransferase; NK, natural killer; Pofut1, protein *O*-fucosyltransferase; SLE, systemic lupus erythematosus; sLe[x], sialyl-Lewis-X; ST3Gal, α2,3-sialyltransferase, TCR, T-cell receptor; TGFβ1, transforming growth factor β1; TLR, Toll-like receptor.