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Glycosylation in health and disease

Colin Reily^{1,5}, Tyler J. Stewart^{2,3,4,5}, Matthew B. Renfrow^{3,*}, and Jan Novak^{2,*}

¹Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA.

²Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA.

³Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA.

⁴National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA.

⁵These authors contributed equally: Colin Reily, Tyler J. Stewart.

Abstract

The glycome describes the complete repertoire of glycoconjugates composed of carbohydrate chains, or glycans, that are covalently linked to lipid or protein molecules. Glycoconjugates are formed through a process called glycosylation and can differ in their glycan sequences, the connections between them and their length. Glycoconjugate synthesis is a dynamic process that depends on the local milieu of enzymes, sugar precursors and organelle structures as well as the cell types involved and cellular signals. Studies of rare genetic disorders that affect glycosylation first highlighted the biological importance of the glycome, and technological advances have improved our understanding of its heterogeneity and complexity. Researchers can now routinely assess how the secreted and cell-surface glycomes reflect overall cellular status in health and disease. In fact, changes in glycosylation can modulate inflammatory responses, enable viral immune escape, promote cancer cell metastasis or regulate apoptosis; the composition of the glycome also affects kidney function in health and disease. New insights into the structure and function of the glycome can now be applied to therapy development and could improve our ability to fine-tune immunological responses and inflammation, optimize the performance of therapeutic antibodies and boost immune responses to cancer. These examples illustrate the potential of the emerging field of ‘glycomedicine’.

* renfrow@uab.edu; jannovak@uab.edu.

Author contributions

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Glycobiology studies the structure, biosynthesis and biology of glycans, which are widely distributed in nature. Most glycans are found on the outermost surfaces of cellular and secreted macromolecules and are remarkably diverse. Simple and highly dynamic protein-bound glycans are also abundant in the nucleus and cytoplasm of cells, where they exert regulatory effects. In fact, in addition to forming important structural features, the sugar components of glycoconjugates modulate or mediate a wide variety of functions in physiological and pathophysiological states¹. Glycoproteins and polysaccharides also have important functions in bacterial cells, and glycoproteins have central roles in the biology of most viruses.

Glycoconjugates are formed by the addition of sugars to proteins and lipids; 17 monosaccharides commonly found in mammalian glycoconjugates are shown in Supplementary Table 1 (REF²). A vast number of naturally occurring sugars can be combined to create a variety of unique glycan structures on lipid and protein molecules that modulate their function. Multiple enzymatic site preferences, as well as the use of stereochemical α or β conjugations, create further diversity in where and how these sugars are linked to each other. In fact, altogether, these features imply the potential existence of $\sim 10^{12}$ different branched glycan structures³. Protein glycosylation includes the addition of *N*-linked glycans, *O*-linked glycans, phosphorylated glycans, glycosaminoglycans and glycosylphosphatidylinositol (GPI) anchors to peptide backbones as well as C-mannosylation of tryptophan residues (FIG. 1). Glycolipids are formed through the addition of sugars to lipids; this type of glycoconjugate includes glycosphingolipids (GSLs)^{4,5} (Fig. 1). Glycosylation of proteins and lipids occurs in the endoplasmic reticulum (ER) and Golgi apparatus, with most of the terminal processing occurring in the *cis*-, *medial*- and *trans*-Golgi compartments. In these organelles, glycosyltransferases and glycosidases form carbohydrate structures in a series of steps that are controlled by substrate availability, enzyme activity, levels of gene transcription and enzyme location within the organelles (Fig. 2). In fact, the glycome of a particular cell reflects its unique gene-expression pattern, which controls the levels of the enzymes responsible for glycoconjugation. Unlike the genome, exome or proteome, the glycome is produced in a non-templated manner and is intricately controlled at multiple levels in the ER and Golgi apparatus.

In this Review, we discuss fundamental concepts in glycobiology and integrate these with recent advances in understanding the key roles of the glycome in health and disease. We review how glycosylation patterns are altered in multiple human diseases, including congenital disorders of glycosylation (CDGs) as well as auto-immune, infectious and chronic inflammatory diseases, and cancer. We also provide specific examples of how an improved understanding of abnormal glycosylation in various diseases might offer new diagnostic options and/or targets for glycan-mediated therapeutic interventions.

Main types of glycosylation in humans

N - linked glycosylation.

Many proteins are modified by *N*-glycosylation, which refers to the attachment of *N*-acetylglucosamine (GlcNAc) to the nitrogen atom of an Asn side chain by a β -1N linkage. These Asn-linked glycoconjugates contain a GlcNAc₂ mannose (Man)₃ core, to which a

variable number of other monosaccharides can be added or removed (FIGS 1,2). These additions include, for example, galactosylation, GlcNAcylation, sialylation and fucosylation, and they determine whether the final structure is classed as a high-mannose *N*-glycan, a hybrid *N*-glycan or a complex *N*-glycan (FIG. 2). *N*-glycans are found in most living organisms and have a crucial role in regulating many intracellular and extracellular functions. *N*-glycosylation depends on the formation of a lipid precursor in which GlcNAc and Man form a branched carbohydrate structure that is attached to dolichol phosphate (Dol-P) on the cytoplasmic side of the ER⁶. This lipid precursor is then flipped to face the ER lumen, where Man and glucose units are added to form a 14-sugar structure — Glc₃Man₉GlcNAc₂ (FIG. 2). Following completion of the Dol-P-carbohydrate structure, an oligosaccharyltransferase adds the carbohydrate chain to a protein at an Asn-X-Ser/Thr site (in which X denotes any amino acid except for Pro); the nascent carbohydrate-protein conjugate undergoes further processing in the ER, which usually involves removal of the glucose residues as part of a quality-control process. The structure then moves to the Golgi apparatus (that is, the *cis*-Golgi), where the carbohydrate structures are trimmed further by a series of specific mannosidases before being transferred to the *medial*-Golgi for maturation. It is within the *medial*- and *trans*-Golgi compartments that hybrid and complex *N*-glycans are produced through the addition of GlcNAc, galactose, sialic acid and fucose sugars⁶ (FIG. 2).

O - glycosylation.

Glycosylation can occur on amino acids with functional hydroxyl groups, which are most often Ser and Thr. In humans, the most common sugars linked to Ser or Thr are GlcNAc and *N*-acetylgalactosamine (GalNAc)⁷ (FIG. 1). GalNAc-linked glycans, often called mucin-type *O*-glycans, are abundant on many extracellular and secreted glycoproteins⁸⁻⁹, including mucins, which form a crucial interface between epithelial cells and the external mucosal surfaces of the body. Mucins are characterized by a variable number of tandem repeats with a high content of Pro, Ser and Thr, which creates many sites for *O*-glycosylation. Moreover, these sites often have extended *O*-glycan cores that create a gel-like substance thought to protect both the glycoproteins and cellular surfaces from external stress, microbial infection and self-recognition by the immune system. This class of *O*-glycans contains six major basic core structures (that is, cores 1–4, terminal GalNAc (Tn) and sialyl-Tn antigens) and is integral, along with other *O*-linked glycoconjugates, to the classification of blood-group antigens (BOX 1). Mucin-type *O*-glycan synthesis is initiated by polypeptide GalNAc transferases (GALNTs)⁷. These GALNTs differ in their specificity for amino acid motifs, although they are often promiscuous, which adds a level of regulation to how and where *O*-glycans are attached. Non-templated sequential addition of glycans to the initial GalNAc gives rise to a diverse set of carbohydrate structures that are often highly clustered on certain glycoproteins, including mucins and human immunoglobulin A1 (IgA1). These sugars are added as the protein moves through the *cis*-, *medial*- and *trans*-Golgi compartments and, unlike *N*-glycans, pre-processing and post-processing that trims existing sugar structures does not occur. Instead, the glycopeptide *O*-glycan chains are modified by distinct glycosyltransferases that can expand the existing structure with galactose, GlcNAc, sialic acid and, in some instances, fucose⁷.

GlcNAc linked to Ser or Thr is typically found on intracellular glycoproteins present in nuclear, mitochondrial and cytoplasmic compartments (FIG. 1). Unlike the mucin-type *O*-glycans, which are GalNAc-linked, addition of GlcNAc does not typically occur in the Golgi apparatus and is not extended; this synthesis is regulated through *O*-linked GlcNAc (*O*-GlcNAc) transferases (OGTs) and *O*-GlcNAcases (OGAs)¹⁰. Although different forms of these enzymes exist depending on the sub-cellular compartment, they all perform the same rapid cycle of addition and removal of GlcNAc from protein substrates. This dynamic process seems to be unique to this glycosylation motif and is thought to regulate many cellular functions, including cellular metabolism. Moreover, *O*-GlcNAc modification competes with protein phosphorylation at Ser and Thr residues, adding to the complexity of regulatory circuits^{11,12}. This functional role of *O*-glycosylation can be controlled by the expression levels of the enzymes involved, as well as substrate concentrations. For the purposes of this Review, other glycoprotein conjugates will not be discussed in detail (reviewed in REFS^{4,13–15}).

Glycosphingolipids.

GSLs comprise a sphingolipid to which a glycan is attached at the C1 hydroxyl position of a ceramide; they are one of the most abundant glycolipids in humans and are typically found in the lipid bilayers of cellular membranes¹⁶ (FIG. 1). GSL glycosylation starts with the addition of glucose or galactose to the lipid moiety at the cytoplasmic side of the ER or the Golgi apparatus, but the structure is then flipped to the luminal side for further processing. The enzymes that initiate GSL glycosylation are specific for lipids, but further processing of the carbohydrate chain can be performed by more general glycosyltransferases. The distribution of different types of GSLs is controlled by functional competition between multiple glycosyltransferases¹⁶. GSLs perform critical cellular functions associated with the formation of lipid rafts, and their glycan composition imparts specific GSL properties¹⁶.

Proteoglycans and glycosaminoglycans.

Proteoglycans are glycoproteins in the extracellular matrix that, in addition to containing canonical *N*-glycans and *O*-glycans, are characterized by the presence of long sugar repeats attached via *O*-linked glycosylation motifs¹⁷. These extended sugar chains are termed glycosaminoglycans and contribute to a substantial proportion of the proteoglycan's molecular mass. Whereas *N*-glycans typically include 5–12 monosaccharides, a glycosaminoglycan motif can easily contain more than 80 sugars (for example, keratan sulfate is a poly-*N*-acetyllactosamine chain that contains up to 50 disaccharide units)¹⁶. These long chains are constructed through disaccharide repeats formed by GlcNAc or GalNAc, combined with an uronic acid (that is, glucuronic or iduronic acid) or galactose. Glycosaminoglycans are functionally diverse and include heparan sulfate, chondroitin sulfate, keratan sulfate and hyaluronan¹⁸. Glycosaminoglycans are crucial to the formation of the glycocalyx, an essential structure for the maintenance of the cell membrane that also functions as a reservoir for sequestered growth factors¹⁸.

Extrinsic glycosylation events.

Extrinsic glycosylation is the process by which soluble glycan-modifying enzymes, such as glycosyltransferases that circulate in the blood, conjugate a monosaccharide to an existing

sugar structure extracellularly^{19–21}. Common acceptor structures for extrinsic glycosylation on mammalian glycoconjugates are galactose (G al)(β 3)-GlcNAc, Gal(β 3)-GalNAc, Gal(β 4)-GlcNAc and GalNAc; β 3 and β 4 represent two different linkage positions on the conjugating sugar^{19–21}. Circulating glycosyltransferases are generated by cleaving the soluble portion of the membrane-associated enzyme and releasing it into the circulation; these soluble enzymes most often originate from liver hepatocytes and platelets. Platelets also contribute to extrinsic glycosylation processes by providing activated sugar intermediates, such as activated sialic acid, galactose and/or fucose^{19–21}. Extrinsic glycosylation processes can generate Lewis and sialyl Lewis antigens, which are fucosylated carbohydrate moieties, as well as Tn and sialyl-Tn antigens, which are *O*-glycans with a terminal GalNAc or sialylated GalNAc, respectively. These glycosylation motifs have an important role in regulating leukocyte trafficking during inflammatory responses and in mediating the interactions between haematopoietic cells and their progenitors in the bone marrow²².

Congenital disorders of glycosylation

Genetic defects in glycosylation are often embryonic lethal, underlying the vital role of glycans^{23–32}; CDGs are classified as type I and type II³². Type I CDGs are caused by abnormalities in the formation of the oligosaccharide structure on the glycolipid precursor before the attachment to the Asn residue of a protein (FIG. 2). Type II CDGs involve defects in the control of the *N*-linked branching structure on the nascent glycoprotein^{30,31}.

CDGs are typically severe in their manifestations, as they affect many muscular, developmental and neurological functions (TABLE 1). In fact, these disorders were originally discovered in children with previously unexplained multi-system disorders; detection of under-glycosylated serum transferrin in the affected children led to the identification of defective glycosylation as the cause for the disease³³. The test used in the diagnosis of CDGs was originally devised to detect alcoholism on the basis of the hyposialylation of liver-derived serum transferrin in patients with alcoholism³⁴. In some CDGs, the defect affects a single glycosylation step or pathway, whereas in other CDGs several pathways are affected. Depending on where the defect in glycosylation occurs, CDG phenotypes can result from altered activation, presentation or transport of sugar precursors; altered expression and/or activity of glycosidases or glycosyltransferases; and altered expression and/or activity of proteins that control the glycosylation machinery or maintain the Golgi apparatus.

N - glycan - related CDGs.

PMM2-CDG is caused by mutations in *PMM2*, which encodes phosphomannanase 2 (PMM2) and is the most common form of CDG; it can present with a neurological or multi-system phenotype^{29,35}. PMM2 converts Man-6-phosphate (Man-6-P) to Man-1-P, a precursor for the synthesis of GDP-(Dol-P-Man). In turn, these compounds are substrates for the mannosyltransferases involved in the synthesis of the lipid-bound precursor of *N*-glycans, Glc₃Man₉GlcNAc₂-P-P-Dol^{29,35}. The type of gene mutation affects the disease severity, which ranges from an embryonic lethal defect if the enzyme is completely inactive

to mild cognitive impairment if the enzyme is still partially active^{29,35}. A long-term follow-up of 75 patients with PMM2-CDG indicated that there were no significant changes in the overall clinical severity over time and that some bio chemical variables spontaneously improved³⁵. Although there are currently no therapeutic options for patients with PMM2-CDG, new treatment strategies that involve the use of pharmacological chaperones to rescue PMM2 loss-of-function mutations are being explored^{36,37}. This strategy is based on the rationale that increasing the stability of mutant enzymes, which often exhibit destabilizing and oligomerization properties, would improve the enzyme activity.

MPI-CDG, the second most common CDG, is caused by mutations in *MPI*, which encodes Man-6-P isomerase²⁹. This enzyme is responsible for the interconversion of Man-6-P and fructose-6-phosphate. Man-6-P can also be generated directly by hexokinase-catalysed phosphorylation of Man, a pathway that is functional in patients with MPI-CDG. Thus, Man dietary supplementation is an effective treatment for MPI-CDG and is well tolerated^{27,38}.

O-glycan-related CDGs.

Congenital muscular dystrophies, such as Walker-Warburg syndrome and muscle-eye-brain disease, involve abnormal Man *O*-glycosylation, primarily on α -dystroglycan³⁹. *O*-Mannosylation of α -dystroglycan and similar proteins is initiated in the ER by the attachment of Man to a Ser or Thr residue, a reaction that is catalysed by the *O*-mannosyltransferase complex that contains POMT1 and POMT2 enzymes^{24–29}. The Man linked to Ser or Thr is then further modified by multiple enzymes in the Golgi apparatus to generate an elongated glycan. The resultant polymeric glycan, matriglycan, is necessary for the binding of laminin and other extracellular matrix proteins to α -dystroglycan^{24,29}. The disruption of this interaction alters the properties of cell membranes and leads to the development of muscular dystrophy. Clinical manifestations of α -dystroglycanopathy frequently include alterations in the central nervous system and ocular disease manifestations, in addition to muscular dystrophy.

NGLY1 deficiency.

NGLY1 deficiency is an autosomal recessive disorder of the ER-associated protein degradation pathway. The level of protein loss (that is, loss of PNGase, encoded by *NGLY1*) correlates with neurological dysfunction, abnormal tear production and liver disease⁴⁰; a nonsense mutation is associated with a particularly severe disease phenotype. PNGase is responsible for the translocation of misfolded proteins across the ER membrane into the cytosol for subsequent degradation by the proteasome⁴¹. A *Drosophila* model of PNGase deficiency led to the identification of various cellular processes associated with PNGase deficiency, including disruption of mitochondrial physiology, reduced cellular respiratory capacity and altered regulation of bone morphogenetic protein^{42–44}. These new insights might lead to novel therapeutic approaches for *NGLY1*-CDG.

Current and future therapies for CDG.

The design or improvement of a diagnostic test, or therapeutic option, for a CDG requires a clear understanding of its pathophysiological mechanisms. Dietary supplementation, such as Man therapy in MPI-CDG, can be very helpful as such approaches are generally

inexpensive and broadly available. However, various dietary supplementation strategies have been tested in multiple CDG s and CDG disease models with varied results. Positive results were observed for dietary supplementation approaches in CAD-CDG, GNE-CDG, PGM1-CDG and SLC 35C1-CDG³⁸. Other CDGs might benefit from a personalized medicine approach based on the identification of relevant genetic mutations in individual patients. Characterizing the underlying mechanism of a CDG at a molecular level should enable the development of new therapeutic approaches.

Glycans in immunity and inflammation

Cells of the immune system, similarly to all other cells, express cell surface-associated glycoproteins and glycolipids that, together with glycan-binding proteins and other molecules, sense environmental signals. Many immune receptors that are expressed on innate and adaptive immune cells recognize glycans found on the surface of microorganisms that are known as pathogen-associated molecular patterns. Examples of such glycan-containing molecules include bacterial lipopolysaccharides, peptidoglycans, teichoic acids, capsular polysaccharides and fungal mannans. The recognition of these glycosylated microbial patterns by the immune system has been exploited for the development of vaccines^{45,46}; pneumococcal vaccines, for example, are formulated using a mixture of capsular polysaccharides⁴⁷. The recent progress in HIV-1 vaccine development has also been driven by a better understanding of the HIV-1 envelope (Env) glycoprotein and the effects of its glycan composition on immune responses and immune evasion^{48–53}. Moreover, the interactions between endothelial cells and leukocytes, which are crucial for leukocyte trafficking and recruitment to sites of tissue injury, are controlled by adhesion molecules that are in turn regulated by cellular glycosylation. Pro-inflammatory cytokines can also induce changes in cell-surface N-glycosylation of endothelial cells, suggesting that glycosylation might contribute to inflammatory vascular diseases^{54,55}.

In the adaptive immune system, glycans also have crucial and multifaceted roles in B cell and T cell differentiation. These functions involve multiple cell-surface and secreted proteins (such as CD 43, CD 45, selectins, galectins and siglecs), different types of cell-cell interactions and the recognition of glycan-containing antigens^{56–58}. The regulation of cellular glycosylation and its impact on the molecules that function as ligands and receptors during an inflammatory response is controlled through various mechanisms and is dependent on the inflammatory insult and its location. These mechanisms, which include ERK and p65 signalling, are critical to understanding the failure to control chronic inflammation in multiple disease states⁵⁵. Immunoglobulins, for example, are crucial components of humoral immunity, and altered glycosylation patterns of some immunoglobulin isotypes have been identified in chronic inflammatory, autoimmune and infectious diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and HIV infection^{59–68}.

In fact, glycosylation patterns differentially affect the effector roles of immunoglobulins^{69–73}. Below, we present several examples to illustrate various biological roles of glycans and glycan-recognizing molecules in B cell and T cell biology, including immunoglobulin effector functions.

CD43 and CD45.

The glycoproteins CD43 and CD45 are abundantly expressed on the surface of B cells and T cells and contain both *O*-glycans and *N*-glycans. Glycosylation of these proteins is modulated during cellular differentiation and activation, and regulates multiple T cell functions, including cellular migration, T cell receptor signaling, cell survival and apoptosis^{74,75}. CD45 has an active receptor-like protein tyrosine phosphatase domain that interacts with Src family kinases in B cells and T cells to regulate the signalling threshold for the activation of B cell receptors (BCRs) and T cell receptors^{75–77}. CD45 also has non-catalytic functions, for example, in modulating the function of the inhibitory co-receptor CD22 on B cells⁷⁸. CD43 is involved in the multiple functions of lymphocytes and other cells of haematopoietic origin, including T cell adhesion and activation. CD43 has an elongated extracellular domain through which it interacts with multiple ligands, including ICAM1(CD54), major histocompatibility class I (MHCI), siglec1 (CD169), galectin 1 and E-selectin. Crosslinking of CD43 with monoclonal antibodies can induce CD43 internalization, whereas phorbol ester-induced activation can lead to both protein internalization and proteolytic cleavage of the CD43 ectodomain; in T cells, CD43 shedding is linked to the regulation of apoptotic cell death⁷⁹.

The glycans present in CD43 and CD45 (namely, their types, size and sites of attachment) are regulated by controlling the expression levels of specific glycosyltransferases and glycosidases. Alternative splicing of the gene that encodes CD45 creates further diversity as it enables the translation of several protein isoforms with different potential glycosylation sites⁷⁴. The affinity of the interactions between glycosylated CD43 or CD45 ligands and their receptors is affected by the presence of core 2 *O*-glycans (that is, GlcNAc β 1–6 (Gal β 1–3) GalNAc.Ser/Thr) versus core 1 *O*-glycans (that is, Gal β 1–3GalNAc.Ser/Thr); the presence of sialic acid is another important factor⁷⁴.

Galectins.

Galectins are small soluble proteins that contain one or two carbohydrate-binding domains specific for galactose-containing glycans⁸⁰. The members of this family of 15 carbohydrate-binding proteins are involved in many processes in the immune system, including the regulation of T cell receptor signal strength as well as T cell and B cell death. For example, galectin 1 can induce apoptosis by binding to *N*-glycans or *O*-glycans present on CD45 (REF⁸¹). Galectin 3 not only interacts with CD43 and CD45 but also binds to highly branched *N*-glycans on extracellular matrix glycoproteins such as laminin, fibronectin, vitronectin and integrin 1, thus affecting cellular adhesion^{80,82}. In addition to binding to extracellular matrix proteins and cell-surface glycol-conjugates in a carbohydrate-dependent manner, galectins can also establish carbohydrate-independent interactions with cytosolic or nuclear targets⁸². Galectins interact with glycopeptides present on the cell surface through the formation of oligomers; oligomerization facilitates receptor clustering, lattice formation and cell-cell interactions. The binding affinity of galectins varies according to the type of glycan it interacts with, and changes in glycan characteristics can regulate the signals induced by galectin binding. For instance, the signal induced by the binding of galectin 1 to CD45 depends on several factors, including the CD45 isoform, which affects its glycosylation potential; the presence of core 2 *O*-glycans, which are high-affinity ligands for

galectin 1; the composition of the *N*-glycan branching; and the extent of sialylation⁸⁰. The addition of sialic acid is dependent on the relative expression of α 2,6-sialyltransferases or α 2,3-sialyltransferases, and the presence of α 2,6-sialic acid on multi-antennary *N*-glycans prevents binding to galectin 1 (REF⁸³). The multiple biological activities of galectins suggest these proteins are valuable potential therapeutic targets in inflammatory diseases and cancer; multiple galectin antagonists are currently under development⁸⁴.

Siglecs.

Siglecs are sialic acid-binding proteins expressed on many cells of the immune system that perform various functions, including the regulation of antigen-specific immune responses and cell homing⁸⁵. CD22 is one of 16 siglec proteins characterized in humans and is expressed on B cells, where it specifically binds α 2,6-linked sialic acid-containing ligands; this interaction is crucial for the formation of nanoclusters in the cell membrane that control BCR signalling following antigen binding⁸⁶. Moreover, CD22 can act as a homing receptor, directing cells to tissues that express high amounts of α 2,6-linked sialic acids⁸⁷. The CD22-CD22L interactions are also essential for maintaining self-tolerance, and CD22-deficient mice produce higher amounts of somatically mutated, high-affinity autoreactive IgG antibodies than wild-type controls⁸⁸. Thus, it seems that CD22 is linked to the tight regulation of BCR signalling that maintains self-tolerance. Perturbations caused by CD22 deficiency might increase the likelihood of developing autoimmune diseases, and CD22 might be a therapeutic target for the treatment of autoimmune diseases such as SLE⁸⁹. Importantly, the success of such a therapy would require a method for targeting CD22-mediated signalling on pathogenic autoreactive B cells without compromising the responses of pathogen-specific B cells.

Another important member of the siglec family is CD169 (also known as sialoadhesin or siglec 1), a macrophage adhesion molecule that binds sialic acid linked by an α 2,3 bond to Gal on *N*-linked and *O*-linked glycans and glycolipids^{90–92}. CD169 binds to sialic acid with low affinity; therefore, its ligands must be heavily sialylated and multimeric to enable an effective interaction⁹³. This siglec mediates cell-cell interactions and the binding of immune cells to sialic acid-containing pathogens. CD169 is also used as a marker for a specific population of macrophages that not only have key roles in the initiation of antibacterial immune responses but are also involved in the transmission of some viruses and in the development of inflammation and several autoimmune diseases^{90,94–96}.

Selectins.

The selectin family of proteins consists of E-selectin, P-selectin and L-selectin, which are mainly expressed on endothelial cells, platelets and leukocytes, respectively; these cell adhesion molecules are critical for leukocyte rolling on the endothelium before tissue extravasation⁹⁷. Selectins recognize sialylated and fuco-sylated glycans but can also bind a subset of heparan sulfate glycosaminoglycans⁹⁸. The finding that inhibition of selectin restored blood flow in a mouse model of sickle cell disease led to a trial of a small-molecule inhibitor of P-selectins and L-selectins, GMI 1070, in patients with sickle cell anaemia; the inhibitor reduced selectin-mediated cell adhesion and abrogated vascular occlusion and 'sickle cell crisis'⁹⁹. Another study demonstrated that inclacumab, a recombinant

monoclonal antibody against P-selectin, reduces myocardial damage after a percutaneous coronary intervention in patients with non-ST-segment elevation myocardial infarction¹⁰⁰. These examples demonstrate that targeting selectins may be beneficial in some inflammatory diseases.

Immunoglobulin glycosylation.

Immunoglobulin isotypes differ in the number of *N*-glycans present on their heavy chains^{62,64,66,73}. Some immunoglobulins, such as IgA1 and IgD, also contain *O*-glycans, which are usually clustered in the hinge-region segments of these antibodies^{101,102}. Immunoglobulin glycans impact the effector functions of antibodies depending on the branching of *N*-glycans and/or the terminal sugars of *N*-glycans or *O*-glycans, which include galactose and sialic acid. In fact, immunoglobulin glycosylation can determine whether an antibody glycoform is pro-inflammatory, such as IgG with galactose-deficient *N*-glycans, or anti-inflammatory, such as IgG with sialylated *N*-glycans.

All four human IgG subclasses have two variable biantennary glycans in their crystallizable fragment (Fc) region, attached at the conserved Asn297 site (FIG. 3a). Certain glycoforms of IgG, such as those deficient in sialic acid and galactose (Fig. 3b), are particularly abundant in some chronic diseases such as RA, SLE, inflammatory bowel disease (IBD), HIV and mycobacterial infections^{59–65}; IgG1 galactose deficiency was also found in individuals with parasitic infection or asthma using population-wide immune activation studies⁶⁶. Biochemical and immunological studies, as well as genome-wide association studies (GWAS), have identified specific genes, enzymes and pathways associated with the production of galactose-deficient IgG molecules; these include β -galactoside α 2,6-sialyltransferase 1, cytokine-signalling adaptor gp 130 and T helper 17 (T_H17) cell-dependent pathways^{67,68}. IgG glycoengineering in vitro⁶⁹ and in vivo⁷⁰ confirmed the pathogenicity of IgGs with galactose-deficient (and therefore, also sialic acid-deficient) *N*-glycans. Conversely, sialylation of pathogenic IgG autoantibodies attenuated their pathogenic activity (Fig. 3c). In fact, *N*-glycosylation of the Fc region of IgG modulates its effector functions as it affects the binding efficiency of Fc γ receptors (Fc γ R s)^{103–105}. On the basis of these and other findings, glycoengineering of therapeutic antibodies and intravenous immunoglobulin (IVIG) has been used to produce therapeutic IgGs with tailored activity, such as sialylated IgG with anti-inflammatory properties^{71–73}.

Human IgA exists in two subclasses, IgA1 and IgA2. Both subclasses have several *N*-glycans in the Fc region, but IgA1 typically also has a cluster of 3–6 *O*-glycans in the hinge region (Fig. 4). IgA and IgM are found in the circulation and in mucosal secretions. Circulating IgA is predominantly monomeric IgA1 (~90%), whereas the remaining IgA is polymeric and consists of two or more monomers connected by a J chain. Secretory IgA is polymeric and includes the secretory component, a heavily *N*-glycosylated polypeptide that is derived from the polymeric immunoglobulin receptor and is added to IgA during transcytosis through mucosal epithelial cells¹⁰⁶. IgA glycans have multiple biological roles, including glycan-mediated antigen-nonspecific binding to bacteria^{102,107}. IgA1 has core 1 *O*-glycans (that is, GalNAc with β 1,3-linked galactose), but in some diseases, including IgA

nephropathy (IgAN), patients have elevated circulatory IgA1 with galactose-deficient *O*-glycans¹⁰⁸.

Glycosylation in cancer

Mechanisms.

Tumour growth depends on the ability of cancer cells to bypass cellular division checkpoints, evade death signals and immune surveillance and migrate to metastatic sites; glycosylation has a role in all of these processes. For example, abnormal growth factor signalling is a critical component of cancer development that can be controlled by the specific glycosylation motifs on the relevant ligands, receptors and molecular scaffolding¹⁰⁹. Glycosylation patterns were one of the first biomarkers of cancer and continue to be used to identify cells with stem-cell-like phenotypes, both within cancer and in healthy tissue^{110–112}. Glycosylation changes that are often observed in cancer cells include an increase in sialyl Lewis structures, abnormal core fucosylation, increased *N*-glycan branching or exposure of the mucin-type *O*-glycan, Tn antigen. Many of these altered glycosylation patterns found in cancer have been termed ‘oncofetal’ as they resemble patterns often seen in early development^{113,114}. As cancer cells evolve through multiple stages of disease, glycan composition can change in parallel with changes in cellular metabolism. This phenomenon includes incomplete synthesis, which refers to truncated glycosylation that produces the Tn antigen in *O*-glycans, and neo-synthesis, which produces abnormal glycosylation patterns such as sialyl Lewis X. These glyco-neoantigens, normally reserved for lymphocyte extravasation from the blood, are commonly found in cancer cells and facilitate metastatic spread^{115,116}.

The number of studies concerning the functional implications of altered glycosylation in cancer has increased over the past decade, but the field still lacks good mechanistic studies of the processes that lead to cancer-associated changes in glycosylation. Proteins from the epidermal growth factor receptor family have both *O*-glycosylation and *N*-glycosylation sites that are modified in many types of cancer through altered expression of glycosyltransferase enzymes, such as polypeptide GALNT3 and Gal 3(4)-L-fucosyltransferase (FUT3). Receptor tyrosine-protein kinase erbB2 (also known as HER2) is overexpressed in a number of cancers, including prostate, breast and gastric cancers, and its functional properties are modulated through a range of complex post-translational modifications, including glycosylation^{117,118}. Loss of GALNT3 can lead to increased production of pro-growth and pro-metastatic carbohydrate antigens, such as T and Tn antigens on HER2, whereas suppression of FUT3 can have an anti-metastatic and growth-suppressing effect by preventing Lewis Y antigen (LeY) production on HER2 (refs^{119,120}). In addition, HER2 can also be regulated through glycoprotein GalNAc 3 β -galactosyltransferase 1 (C1GalT1) activity, as increased mucin-type *O*-glycosylation enhances galectin 4 binding, which leads to HER2 activation^{118,121}. Abnormal promoter region methylation in some glycosyltransferase genes, such as *GALNT3*, can also lead to aberrant enzyme expression¹²².

Currently, there is a dearth of studies in the cancer field that assess the intracellular mechanisms that drive changes in expression and activity of specific glycosyltransferases

with respect to their targets. An exception is mucin 1 (MUC1), one of the most studied glycoconjugates in the cancer field, partly owing to its increased expression and altered glycan composition in many adenocarcinomas, and partly owing to its use as a cancer-vaccine antigen^{123–125}. MUC1 contains a large extracellular domain (that is, the mucin domain) with five potential *O*-glycosylation sites in each of its multiple repeats that consist of ~20 amino acid residues. Studies of glycosyltransferase enzymes implicated the dysregulation of several enzymes in altering *O*-glycosylation of MUC1, including C1GalT1, β -galactoside α -2,3-sialyltransferase 1 (ST3GalI) and α -GalNAc α -2,6-sialyltransferase 1 (ST6GalNAcI); altered localization of GALNTs in the ER also has a role in changing MUC1 glycosylation^{126–129}. GALNTs typically initiate *O*-glycosylation in the Golgi apparatus, but in cell-culture models these enzymes can translocate to the ER via a process that involves aberrant Src signalling, leading to an increased density of *O*-glycosylation of MUC1 repeats^{128,130}; MUC1 produced by cancer cells also exhibits increased sialyl-Tn antigen^{129,131,132}. In addition, altered glycosylation motifs on MUC1 can affect cancer immune surveillance, typically through co-opting cell-surface lectins such as CD169, which enhances macrophage activation after binding to sialylated MUC1 and promotes tumour growth^{133–135}.

The glycosylation of specific proteins in serum and/or tumour tissues can be used as a diagnostic biomarker and to assess patient prognosis and responses to treatment; examples of such glycoproteins include CEA, MUC1, MUC16 and prostate-specific antigen (PSA)^{136–141}. Carbohydrate antigen 19–9 (CA19–9) is detected with a specific mouse monoclonal antibody that recognizes the sialyl Lewis A (sLea) carbohydrate motif (that is, Neu5Ac α 2,3Gal β 1,3(Fuc α 1,4)GlcNAc β 1-R) on a monosialoganglioside first identified in gastrointestinal cancer¹⁴². Synthesis of this sLe^a antigen is controlled by Lewis, a gene involved in ABO(H) blood-group determinants¹⁴³ (Box 1). CA19–9 is often, but not always, elevated in the serum of patients with a variety of cancers, including pancreatic, gastric and colorectal cancers; the mechanisms that lead to this increase in serum levels in cancer are poorly understood, but it seems to be associated with dysregulated sialyltransferases, such as Gal- β -1,3-GalNAc- α -2,3-sialyltransferase 2 and 4 (ST3GalII and ST3GalIV, respectively)¹⁴⁴. The presence of CA19–9 across multiple cancers highlights abnormal glycosylation as a fundamental feature of cancer pathobiology^{136,143}.

Another important glycosylation pattern is the α 2,6-linked sialic acid in *N*-glycans, which is commonly elevated in pancreatic and colon cancers and has pro-tumour effects; this sialic acid is added to the glycan backbone by β -Gal α -2,6-sialyltransferase 1 (ST6GalI), also upregulated in these cancers^{145–147} (Fig. 5). Increased expression of ST6GalI is associated with pro-survival pathways, and its sialylated products can inhibit tumour cell apoptosis and activate growth factor pathways^{110,111,148–150}.

Glycome profiles.

Although the use of a single biomarker for the diagnosis and monitoring of disease progression is an attractive prospect, research shows that whole glycome profiles might be better than a single glycosylation pattern for the assessment of disease progression. In one study, glycome profiling of prostate cancer biopsy samples enabled the identification of

indolent versus metastatic disease with 91% accuracy; conventional assessment was 72% accurate¹⁵¹. Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) of hepatocellular carcinoma biopsy samples identified increases in fucosylation across a panel of *N*-glycosylation structures in 95% of patients; the specific glycome profiles correlated with median patient survival time¹⁵². Transcriptional analysis of melanoma biopsy samples indicated that expression of β 1, 6-*N*-acetylglucosaminyltransferase (GCNT2) in these samples was decreased compared with those of healthy epidermal melanocytes. This decrease led to a decrease in Asn-linked I-branched glycans (that is, glycans that have additional GlcNAc-Gal branches, also termed 'adult I' blood-group antigen), a novel marker of metastatic melanoma progression¹⁵³. Collectively, these studies highlight the advantages of whole glycome profiling over the detection of traditional single markers of glycosylation in cancer.

Xenotransplantation

The major bottleneck for organ transplantation continues to be the shortage of available viable organs. Xenotransplantation (for example, from pig donors) is a proposed solution for this problem¹⁵⁴ but it has been hindered by several factors, including the presence of donor carbohydrate antigens that are not present in humans and can trigger immune rejection. Examples of such antigens include the Gal- α (1,3)-Gal epitope present on glycoproteins and glycolipids and *N*-glycolylneuraminic acid (Neu5Gc).

The Gal- α (1,3)-Gal epitope is synthesized by an α 1,3-galactosyltransferase in New World primates and many non-primate mammals. However, the gene that encodes this enzyme, *GGTA1*, is inactivated in humans and Old World primates, which leads to the production of antibodies that recognize Gal- α (1,3)-Gal-containing glycoconjugates¹⁵⁵. It is thought that these antibodies are induced by Gal- α (1,3)-Gal-containing compounds present in the microbiota and from dietary sources. The antibodies bind to vascular Gal- α (1,3)-Gal antigens of xenotransplants and induce complement-mediated endothelial cell cytotoxicity that results in graft rejection¹⁵⁶. Importantly, deletion of *GGTA1* from the pig genome revealed the presence of additional endothelial cell xenoantigens that could contribute to graft rejection¹⁵⁶.

Neu5Gc is another relevant xenoantigen, and it is synthesized by CMP-*N*-acetylneuraminic acid hydroxylase. This enzyme is present in most mammals, including pigs, but it is inactive in humans, although Neu5Gc can be obtained from dietary sources and metabolically incorporated into many human glycoconjugates^{157,158}. Neu5Gc, also termed Haganutzui-Deicher antigen, has been identified as the antigen in horse serum that causes serum sickness in humans¹⁵⁹. A third example of an important xenoantigen found in pig tissues is terminal GalNAc, also known as the SDA blood-group antigen, which is synthesized by β 1,4-*N*-acetylgalactosaminyltransferase 2, encoded by *B4GALNT2*. Although SDA^a is expressed in various human cells, anti-SDA^a IgM is commonly detected in human serum¹⁶⁰.

It is hoped that modern approaches based on CRISPR-Cas-mediated gene editing might be used to remove these xenoantigens and aid the development of animal tissues and organs that are suitable for transplantation in humans^{155,157}.

Autoimmunity and chronic inflammation

The immune system routinely recognizes and responds to foreign carbohydrate epitopes, such as lipopolysaccharides; however, loss of tolerance and autoimmunity might also occur, for example, if the glycosylation patterns of the host and the pathogen overlap, perhaps owing to abnormal enzymatic activity. Changes in the glycosylation patterns of proteins can also result in immune detection of these neo-glycan epitopes and lead to autoimmunity. In addition, IgG effector functions are controlled by *N*-glycosylation. Altered sialylation, galactosylation and/or fucosylation, as well as changes in glycan composition, can contribute to immune dysregulation and a range of autoimmune and chronic inflammatory diseases.

Rheumatoid arthritis.

RA is an autoimmune disease that results in chronic inflammation of joints and other associated tissues; it is characterized by the presence of serum rheumatoid factor and anti-cyclic citrullinated peptide antibodies¹⁶¹. Early studies of collagenous tissues from patients with RA found considerable immune aggregates consisting of IgG and IgA¹⁶². Moreover, IgG from patients with RA has overall and site-specific changes in glycosylation that affect the composition of Fc-associated and antigen-binding fragment (Fab)-associated glycans. In addition to an increased proportion of Gal-deficient Fc glycans, Fab-portion glycans contain high amounts of bisecting GlcNAc and core fucose¹⁶³. Changes in the glycosylation of antigen-specific IgGs precede disease onset, and the degree of these changes correlates with disease severity^{164,165}. Altogether, these findings suggest that changes in the glycosylation of IgG are a critical component of RA pathogenesis.

Inflammatory bowel disease.

IBD refers to chronic inflammatory diseases that affect the gastrointestinal tract and includes Crohn's disease and ulcerative colitis. In IBD, both decreased secretion of mucins and structural changes to mucins themselves can occur; the mucins of the gastrointestinal tract form a physical barrier between the intestinal microbiota and the intestinal epithelium¹⁶⁶. Chronic inflammation in the gastrointestinal tract is thought to involve gut microbiota-induced changes in the glycosylation patterns of the host that result in enhanced entry of bacteria or dietary lectins into the host tissue^{167–170}. This process leads to increased inflammation that can lead to ulcer development and cancer¹⁷¹. In addition to potential changes in glycosylation associated with alterations in gut mucosa and the epithelium, changes in *N*-glycosylation of IgG are also observed. Patients with IBD have decreased galactosylation of IgG *N*-glycans, and the extent of under-galactosylation correlates with disease severity⁶⁰. Specific changes in antibody glycosylation could be used to discriminate between patients with ulcerative colitis and those with Crohn's disease¹⁷².

Systemic lupus erythematosus.

SLE is a systemic autoimmune disease characterized by the presence of polyreactive autoantibodies that bind to different host targets, including proteins, nucleic acids and their complexes. Similarly to other autoimmune diseases, reduced galactosylation and sialylation of IgG are associated with SLE^{63,173}. Furthermore, a decrease in core fucosylation and an

increase in bisecting GlcNAc of *N*-glycans is observed. Together, these findings show that alterations in the IgG glycome in SLE limit the negative feedback loops that rely on complete IgG glycosylation and promote an inflammatory glycan profile. It is unknown whether the changes in glycosylation of IgG associated with SLE correlate with a loss of B cell tolerance and the production of autoreactive antibodies. Interestingly, treatment with sialylated IgG protected against disease symptoms in a mouse model of SLE¹⁷⁴.

Tn syndrome.

Originally called permanent mixed-field polyagglutinability, Tn syndrome is a rare blood disorder characterized by the presence of Tn antigens on all haematopoietic cell lineages¹⁷⁵. In this syndrome, the Tn antigen-containing glycoconjugates are recognized by IgM antibodies, which leads to varying degrees of anaemia, leukopenia and thrombocytopenia in affected patients. Tn syndrome is a disorder of *O*-glycosylation in which Tn antigen is over-represented owing to a decrease in the galactosylation of terminal GalNAc by C1GalT1. This syndrome is an acquired and permanent disease that can occur in males and females at any age¹⁷⁵ and results from a clonal somatic mutation in *COSMC* (also known as *CIGALT1C1*), which encodes a molecular chaperone specific for C1GalT1 (refs^{176,177}). Mutations in *COSMC* reduce the amount of active C1GalT1, thus decreasing GalNAc galactosylation.

Granulomatosis with polyangiitis.

Patients with granulomatosis with polyangiitis (GPA; formerly called Wegener granulomatosis) often have circulating IgG anti-neutrophil cytoplasmic antibodies (ANCA), typically specific for proteinase 3; these antibodies are thought to activate resident and/or infiltrating macrophages in the vasculature, leading to inflammation. GPA affects small-to-medium-sized blood vessels; therefore, the lung and kidney are particularly vulnerable to macrophage-mediated damage. Several studies reported that abnormal glycosylation of the IgG Fc region (namely, low galactosylation and sialylation) correlates with driving the enhanced macrophage activation. Anti-proteinase 3 IgG antibodies, which are significantly elevated in patients with GPA, are less sialylated than IgG from healthy individuals^{178–180}. These hyposialylated autoantibodies lead to enhanced macrophage activation when compared with antibodies with normal sialylation, and increased levels of hyposialylated autoantibodies are associated with disease activity^{178–181}.

IgA nephropathy.

IgAN is a unique autoimmune disease in which the autoantigen is an antibody itself — specifically, IgA1 antibodies that contain galactose-deficient *O*-glycans and terminal GalNAc¹⁸² (Fig. 6). GWAS^{183,184} and biochemical studies^{185,186} identified an association between several glycosyltransferases, and related proteins, with galactose deficiency of IgA1 in IgAN. In fact, patients with IgAN have elevated levels of serum IgA1 with altered mucin-type *O*-glycans in the hinge region; serum levels of galactose-deficient IgA1 and the corresponding autoantibodies are predictive of disease progression¹⁸² (Figs 4,6). Disease pathogenesis is not completely understood but is thought to involve altered expression and activity of key enzymes in IgA1-secreting cells, which, in genetically susceptible individuals, lead to the production of IgG autoantibodies. Consequently, circulating immune

complexes form in blood, some of which deposit in the mesangium and activate mesangial cells, leading to renal injury; approximately half of patients with IgAN progress to end-stage renal disease¹⁸² (Fig. 6).

In IgAN, several factors might contribute to increased levels of Tn and sialylated Tn (sTn) antigens on IgA1, including decreased expression of C1GalT1 or C1GalT1C1 as well as increased expression of ST6GalNAcII, leading to premature sialylation of GalNAc¹⁸⁵ (Fig. 6). Alternatively, changes in the activity of GALNTs might alter the sites and/or densities of initial GalNAc attachment and thereby contribute to the formation of Tn and sTn antigens. Technological advances in glycoconjugate analysis have enabled researchers to recognize that there is restricted heterogeneity in the glycosylation pattern of the IgA1 hinge region, which not only varies between patients but can also vary within individuals¹⁸⁷ (Box 2; Supplementary Table 2). Together, these mechanisms demonstrate the critical role of correct glycan composition during glycoprotein production and demonstrate that altered glycosylation can result in the presentation of glycan autoantigens and the onset of autoimmunity.

Diabetes mellitus.

Diabetes mellitus is commonly characterized by excess glucose in the blood, which predictably leads to various glycosylation abnormalities in patients with both type 1 and type 2 diabetes. The most widely used marker for monitoring the long-term management of diabetes is glycated haemoglobin (HbA1c), which is a surrogate for the average blood glucose levels in the previous 3 months^{188–190}. Non-enzymatic glycosylation (also known as glycation) based on reactions between haemoglobin and glucose can produce multiple glycoforms, but, in the case of HbA1c, it requires a multistep reaction between the amino-terminal Asp on the haemoglobin β -chain and a condensation reaction at the 1-hydroxyl of the glucose molecule followed by an Amadori rearrangement^{191–193}. A similar non-enzymatic glycosylation process produces advanced glycation end products, which are glycated proteins and lipids that are elevated in diabetes and are linked to disease pathology^{194,195}.

O-GlcNAc glycosylation has an important role in many cellular control mechanisms in general and in diabetes specifically, including the cellular response to insulin¹⁹⁶; it is catalysed by OGT, which adds GlcNAc to Ser and/or Thr residues^{197–199}. OGT uses UDP-GlcNAc as a substrate, which is a product of the hexosamine biosynthesis pathway. This pathway functions in part as a glucose sensor and regulates cellular responses to insulin by controlling the levels of UDP-GlcNAc-mediated glycosylation of targets related to insulin activity^{200,201}. The hexosamine biosynthesis pathway is highly responsive to glucose levels, and its flux is significantly increased in some tissues of patients with diabetes, leading to increased levels of UDP-GlcNAc and, thus, elevated *O*-GlcNAc glycosylation^{202–204}. For example, *O*-GlcNAc glycosylation of the transcription factor NeuroD1 is crucial for its nuclear translocation, which leads to insulin production by pancreatic β -cells in response to high glucose levels²⁰⁵. Long-term exposure to high glucose increases *O*-GlcNAc modifications and, thus, its biological effects; in the context of diabetes, increased *O*-GlcNAc glycosylation of proteins, such as AKT, can lead to enhanced β -cell death^{206,207}.

Cardiomyocytes can be damaged by high blood glucose levels, leading to the high rates of systolic or diastolic dysfunction in patients with diabetes (~16.9%)²⁰⁸; in animal models of diabetes, contractility deficits in the heart are associated with *O*-GlcNAc glycosylation levels²⁰⁹. In a rat model of streptozotocin-induced diabetes, ventricular arrhythmias were associated with *O*-GlcNAc modification of the sodium channel protein type 5 subunit- α (HH1). Decreasing *O*-GlcNAc addition to HH1 during high-glucose stress improved the function of this sodium channel²¹⁰. In addition, using an adenovirus injection to the heart to increase the expression of protein OGA, the enzyme that removes *O*-GlcNAc adducts, improved cardiomyocyte contractility and decreased *O*-GlcNAc-protein levels in the same model of diabetes²¹¹. However, in a model of myocardial ischaemia–reperfusion injury, decreased *O*-GlcNAc-protein levels were associated with increased infarct size in diabetic mice when compared with controls. Recovery of *O*-GlcNAc levels via miR-24 activation of OGT prevented the increase in infarct size in diabetic mice²¹². In the context of cardiac function, appropriate regulation of *O*-GlcNAc glycosylation is crucial as, depending on the cardiac insult, glycosylation that is either increased by OGT or reduced by OGA might be beneficial.

N-glycosylation defects can also cause diabetes, as demonstrated by a mouse model in which inactive *N*-acetylglucosaminyltransferase-IVa (GnT-IVa) led to impaired insulin release and hyperglycemia²¹³. A mass spectrometric analysis of glycosylated proteins in the kidneys of two mouse models of diabetes, caused by leptin receptor-deficiency (db/db) or by administration of streptozotocin, showed significant differences in general protein *N*-glycosylation between diabetic mice and healthy controls, with some similarities between the two models of disease²¹⁴. Another study of serum samples from 818 patients with diabetic kidney disease reported that HbA_{1c} positively correlated with the degree of complex and branched *N*-glycan content on IgG²¹⁵. In addition, hyposialylated IgG, which is often present in the serum of patients with type 2 diabetes²¹⁶, activates endothelial Fc γ RIIIb, which leads to insulin resistance in obese mice. Further studies are required to understand the mechanisms underlying *N*-glycosylation changes in diabetes.

Glycans and glomerular filtration

Glycans have many roles in physiological kidney function, including several major roles in glomerular filtration. The most relevant glycoconjugates are found in the glycocalyx of the fenestrated glomerular endothelial cells, in the glomerular basement membrane (GBM), formed by extracellular matrix proteins, and in the podocyte slit diaphragm (Fig. 7).

The first barrier that blood encounters in the glomeruli is the fenestrated endothelium, which determines the glomerular filtration rate^{217,218}. The fenestrated endothelium also acts as a crucial barrier that prevents proteins from entering Bowman's space; however, the pore size of the fenestrated endothelium cannot alone prevent albumin and other macromolecules from entering Bowman's space^{217,218}. Rather, the endothelial glycocalyx, which covers the endothelial fenestrae, probably contributes to the high permselectivity of glomeruli²¹⁹. The glycocalyx is composed of negatively charged glycoproteins, glycosaminoglycans and membrane-associated and secreted proteoglycans that form an interlinking network of proteins and glycans.

One of the most evident roles of glycans in renal function relates to the formation and maintenance of the GBM, which connects the basal lamina of the fenestrated endothelial cells to the foot processes of podocytes. The GBM consists of three layers: the lamina rara interna, which is adjacent to the endothelial cells and is composed of negatively charged heparan sulfate proteoglycans; the lamina densa, a dark central zone composed of collagen IV and laminin; and the lamina rara externa, adjacent to podocyte foot processes and composed of heparan sulfate proteoglycans. The heavily sulfated glycans of proteoglycans, such as agrin and perlecan, are responsible for the negative charge of the GBM, which may have a role in regulating complement activation in the glomeruli²²⁰. Loss of heparan sulfate chains from agrin is observed in several glomerulopathies, including lupus nephritis, membranous nephropathy, minimal change disease and diabetic kidney disease, suggesting a prominent role for heparan sulfate in GBM function²²¹. Although multiple mechanisms might account for this decrease in heparan sulfate, increased activity of heparanase, an endo- β (1–4)-D-glucuronidase that degrades heparan sulfate, has been observed in both rodent models and patients with diabetic kidney disease, minimal change disease, membranous nephropathy and IgAN^{222,223}; heparanase treatment dramatically increases the permeability of the GBM²²⁴. In addition to heparan sulfate, O-mannosyl glycans of α -dystroglycan mediate the interface between podocyte foot processes and the GBM through direct interactions with agrin and laminin 11 (ref.²²⁵). Deglycosylated α -dystroglycan can no longer bind agrin, which might lead to the detachment of podocytes from the GBM and impaired glomerular filtration in vivo²²⁶.

Glycans also have important roles in the formation of the slit diaphragm, a specialized intracellular junction between adjacent podocyte foot processes that is formed by a complex network of proteins and mediates intracellular and extracellular interactions²²⁷. Nephrin is a membrane glycoprotein expressed on the surface of podocytes at the intersection of podocyte–podocyte foot processes²²⁸; it functions as an adhesion and scaffolding receptor and a signalling molecule. N-glycosylation of up to ten potential glycosylation sites is critical for the appropriate folding, transport, surface expression and function of nephrin^{229,230}. In fact, N-glycan defects in nephrin result in poorly formed slit diaphragms and compromised kidney function^{231,232}.

Mutations in a key enzyme involved in sialic acid biosynthesis, bifunctional UDP-N-GlcNAc 2-epimerase/N-acetylmannosamine (ManNAc) kinase, cause glomerular proteinuria in mice, which can be rescued with ManNAc administration. Analysis of renal tissue sections from these mice showed segmental splitting of the GBM and effacement of podocyte foot processes as well as decreased sialylation of O-glycans in the podocalyxin protein²³³. Podocalyxin is a sialoglycoprotein and a major constituent of the glycocalyx of podocytes in the glomerulus, where the negatively charged sialic acid is believed to separate adjacent foot processes and retain space for glomerular filtration²³⁴. In vivo, removal of α 2,6-linked sialic acid from the glomerular filtration barrier results in proteinuria and renal failure, along with the apparent formation of irreversible tight junctions between adjacent podocytes²³⁵.

Glycomedicine: glycans in therapeutics

Developments in the field of glycobiology have enabled the development of a variety of glycan-based therapeutics (Table 2). For example, envelope glycoprotein gp¹²⁰ is expressed on the surface of HIV-1, and its variable glycosylation facilitates viral escape from immune detection^{236,237}. Adding new glycan-dependent epitopes to the recombinant gp120 used for vaccination increased the ability of broadly neutralizing monoclonal antibodies to recognize HIV-1, suggesting that this approach can be used to optimize vaccination protocols and antigens^{238,239}. Moreover, HIV-1 envelope glycoproteins not only differentiate HIV-1 clades but can also be used to estimate the efficacy of vaccine regimens on the basis of antibody binding to a panel of gp120 glycan-dependent epitopes²⁴⁰.

As outlined previously, glycosylation plays a critical role in regulating functional immune responses through complex receptor–glycan motif interactions. This aspect is now being exploited in immunoglobulin therapies^{241–243}. One study found that IgG can create hexameric structures on the cell surface to increase Fc-mediated complement activation²⁴⁴. This information led to the creation of an hexamer composed of fused Fc segments from IgG; this multimer, termed Hexa-Fcs, enhanced Fc receptor avidity, which is co-dependent on IgG glycosylation. Furthermore, addition of new *N*-glycosylation sites to Hexa-Fcs increased the overall sialic acid content, which could potentially enhance its immunomodulatory function²⁴⁵. Specifically, enhanced sialylation of IgG Fc glycans decreases complement-mediated cytotoxicity by reducing C1q binding to IgG1 Fc²⁴⁶; these observations have important implications for the treatment of patients using IVIG therapy.

Glycan-dependent IgG effector functions are recognized as critical components of therapeutic antibodies in cancer²⁴⁷, which are now tailored through glycoengineering. For example, removal of fucose increases antibody-dependent cell-mediated cytotoxicity, which is very important for certain antibodies used to treat patients with cancer^{248,249}. Increased galactosylation of the Fc in rituximab, an anti-CD20 antibody, enhances complement-dependent cytotoxicity^{250,251}. Moreover, IgG with elevated content of α 2,6-linked sialic acid has an anti-inflammatory effect, a property that could be exploited for the treatment of autoimmune diseases^{252,253}. This anti-inflammatory activity is dependent on binding of CD209 (also known as DC-SIGN) on dendritic cells to sialylated IgG, which leads to the upregulation of Fc γ RIIb and regulatory T (Treg) cell expansion^{252,253}. In addition to the modification of *N*-glycosylation motifs, the effector function of antibodies that lack glycosylation (that is, aglycosylated IgG) is also being investigated²⁵⁴. Some examples of aglycosylated biologics are mogamulizumab, an anti-CD¹⁹⁴ for treatment of T cell lymphoma; otelixizumab, an anti-CD3 for the treatment of type 1 diabetes; and MTRX-1011 A, an anti-CD4 for the treatment of RA^{249,255–258}.

The therapeutic use of sugars, or drugs that directly target glycosylation, has eluded clinical application, with the exception of the use of heparan to prevent blood clotting and the use of nutritional supplements to alleviate the symptoms of certain CDGs, as discussed earlier. Targeting the glycosylation pathways of galectin 1 with drugs such as anginex²⁵⁹ and OTX001 (ref.²⁶⁰) has also been tested for the reduction of angiogenesis in cancer; preliminary results showed a modest decrease in angiogenesis after treatment with anginex,

whereas OTX001 had a better effect and is now in phase I clinical trials. Ex vivo treatment of T cells from patients with ulcerative colitis with addition of GlcNAc increased levels of *N*-glycosylation branches on the T cell receptor and led to a decrease in pro-inflammatory cytokines²⁶¹. Thioglycosides, which are S-glycoside GlcNAc mimetics, have been studied for their anti-inflammatory effects along with *O*-glycosides and shown to significantly decrease sialyl Lewis X levels on endothelial cells and E-selectin-induced leukocyte rolling²⁶². In vitro, thioglycosides also blocked SGLT1 and SGLT2 (also known as SLC5A1 and SLC5A2, respectively), which are targeted in diabetes to prevent the reabsorption of glucose in the kidney²⁶³.

An animal model of minimal change disease revealed that angiopoietin-related protein 4 (ANGPTL4) is hyposialylated and has a causative role in proteinuria. Oral treatment with the sialic acid precursor, ManNAc, rescued the hyposialylation of ANGPTL4 and prevented proteinuria^{264,265}. In a mouse model of unilateral ureteral obstruction, glucosamine hydrochloride supplementation protected against renal fibrosis; glucosamine hydrochloride decreased N-glycosylation of transforming growth factor- β (TGF β) receptor type 2 (TGFR2), the receptor for TGF β 1, thus inhibiting its translocation to the cell surface membrane and initiation of pro-fibrotic pathways²⁶⁶. Most therapeutics that target glycosylation pathways and/or synthetic saccharides for the treatment of non-CDG pathologies are still at an experimental stage, but they hold considerable promise given the key role of glycosylation in numerous pathobiologies.

Conclusions

Glycosylation is a common modification of proteins and lipids that involves non-templated dynamic and complex processes. Glycans have multiple crucial roles in cellular responses to environmental stimuli as well as cellular growth and differentiation; specific changes in glycan composition are directly linked to many diseases. Technological advances are beginning to overcome many of the challenges posed by the complexities of glycoconjugates, improving our understanding of the physiological and pathological processes that are regulated by glycans (Box 2). Such efforts are further supported by improvements in research tools as well as training in glycosciences^{1,267}, both of which facilitate the advancement of glycomedicine, in which glycobiology is applied to the development of novel therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Glycans

Saccharides or sugar chains that can be free or attached to proteins or lipids to form simple or complex glycoconjugates

***N*-linked glycans**

Branched protein glycans attached through a nitrogen atom of Asn residues at Asn-X-Ser/Thr motifs

***O*-linked glycans**

Diverse protein glycans typically attached to an oxygen atom of Ser or Thr residues

Glycosaminoglycans

Long unbranched polysaccharides made of repeating disaccharide units often attached to proteins

Glycosylphosphatidylinositol (GPI) anchors

Short glycolipids that link proteins to the cell membrane

C-mannosylation

The attachment of an α -mannopyranosyl to the indole-C2 carbon of a Trp residue

Glycosphingolipids

(GSLs). Sphingolipids with attached glycan moieties that exist in the cell membrane

Glycosyltransferases

Enzymes that add glycans

Glycosidases

Enzymes that remove glycans

High-mannose *N*-glycan

A less-processed *N*-glycan with high mannose content

Hybrid *N*-glycan

A partially processed *N*-glycan with mannose and one antenna containing *N*-acetylglucosamine

Complex *N*-glycan

Processed *N*-glycan with two, three or four antennas and possibly with bisecting *N*-acetylglucosamine

Basic core structures

For *N*-glycans, the basic core structure is a common pentasaccharide GlcNAc₂Man₃. For *O*-glycans, cores 1–4 are defined on the basis of the glycans attached to the initial *N*-acetylgalactosamine (GalNAc); for example, core 1 is GalNAc with p 1,3-linked galactose

Glycocalyx

A cell-surface layer of glycosaminoglycans, proteoglycans and glycoproteins that extends far from the cell membrane

Activated sugar intermediates

Monosaccharides with high-energy donors attached, such as UDP-sugar, GDP-sugar or, in the case of sialic acid, CMP-sialic acid

Dandy-Walker malformation

A brain malformation that occurs during embryonic development of the cerebellum (linked to movement, coordination, cognition and behaviour) and the fourth ventricle (which channels fluid from inside to around the outside of the brain)

Coloboma

An eye abnormality that occurs before birth and refers to missing pieces of tissue in structures that form the eye, such as the iris, retina, choroid or optic disc

Multi-antennary *N*-glycans

N-linked glycans with multiple branching glycan structures (that is, three or more antennas)

J chain

Joining chain that links monomers of immunoglobulin A (IgA) or IgM to form polymeric IgA or polymeric IgM

Monosialoganglioside

A ganglioside, which is a ceramide with a carbohydrate motif, that contains a single sialic acid

Rheumatoid factor

Autoantibodies specific for the crystallizable fragment (Fc) of immunoglobulin G (IgG) that are often present in the blood of patients with rheumatoid arthritis

Condensation reaction

A reaction in which two molecules combine, releasing an H₂O in the process

Amadori rearrangement

A rearrangement reaction catalysed by a base or acid on the *N*-glycoside of an aldose to a 1-amino-1-deoxy-ketose

Hexosamine biosynthesis

Nutrient-sensing pathway that converts fructose-6-phosphate to UDP-*N*-acetylgalactosamine.

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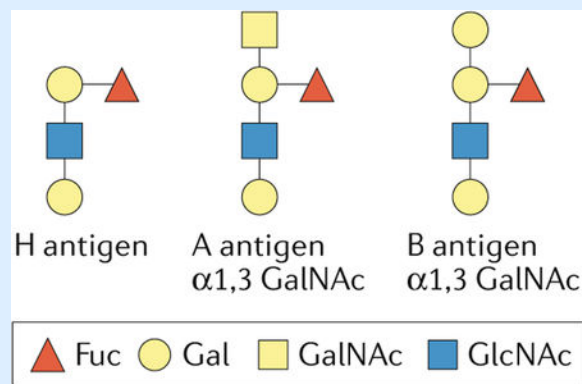
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Key points

- Glycosylation is critical for physiological and pathological cellular functions; advances in analytical techniques have driven progression in the field of glycobiology over the past decade.
- Congenital disorders of glycosylation have provided considerable insight into basic mechanisms underlying the associations of specific glycoconjugates with disease phenotypes.
- Interactions between immune cells that are mediated by cell surface molecules and drive cellular activation are regulated by the glycosylation motifs of membrane-bound glycoconjugates and their binding to sugar-specific receptors.
- Cancers often exhibit oncofetal phenotypes that are reflected in the nature of their glycoconjugates; these changes in glycosylation drive metastatic properties, inhibition of apoptosis and resistance to chemotherapy.
- The pathogenesis of many autoimmune diseases, such as immunoglobulin A (IgA) nephropathy, systemic lupus erythematosus and inflammatory bowel disease, involves abnormal glycosylation of one or more glycoproteins; diabetes involves abnormal *O*-linked *N*-acetylglucosamine-mediated signalling and enhanced glycation of multiple proteins.
- Immunoglobulin glycosylation controls the effector functions of antibodies, which creates opportunities for the therapeutic application of glycoengineering.

Box 1 |**Blood-group sugars can be immunogenic**

Many blood-group antigens on erythrocytes are glycans conjugated to lipids or proteins. The ABO(H) blood-group antigens were discovered in the early 20th century on the basis of the existence of antibodies in some individuals that agglutinated red blood cells from other individuals. Follow-up studies revealed that these antibodies recognized glycan epitopes on the cell surface of red blood cells that were also expressed in other cells of the same individual. These studies established for the first time that glycans can be antigenic. The ABO(H)-associated glycan epitopes are determined by the inheritance of glycosyltransferase genes in ABO, *FUT1* and *FUT2* loci. Group O individuals express the H antigen, the precursor to A and B antigens. Group A individuals have α 1,3-*N*-acetylglucosamine (GlcNAc) attached to the galactose residue of the H antigen, whereas Group B individuals have α 1,3-Gal. Additional blood-group antigens have been described, and many contain glycan epitopes such as Lewis antigen. Compatibility of ABO(H) and other antigens between donor and recipient is necessary for a successful blood transfusion^{268,269}. Some blood-group antigens affect the binding of bacteria and viruses to host cell surfaces and represent risk factors in chronic inflammatory diseases^{270–273}. For example, the blood-group O phenotype is associated with disease severity in patients infected with *Vibrio cholerae*²⁷⁴. Human noroviruses, which cause sporadic and epidemic gastroenteritis, also selectively bind ABO(H) and Lewis blood-group antigens²⁷⁵; some viral strains recognize the A and/or B and H antigens, whereas others recognize only the Lewis and/or H antigens. Thus, susceptibility to these viruses is influenced by the host's blood-group antigens and the sequence variations of the capsid protein²⁷⁶.



Box 2 |**Techniques for glycoconjugate analysis**

Over the past two decades, many different technologies have been developed for the analyses of glycans and glycoconjugates. Many of the initial glycan analyses were carried out using lectins that recognize a specific glycan or glycans, often combined with pretreatment with glycosidases to selectively remove monosaccharide residues or larger components of extended glycoconjugates. For more detailed analysis, mass spectrometric techniques are now commonly used for global and individual molecular analyses. The advent of chemo-enzymatic techniques for the synthesis of glycans has driven the more widespread use of glycan array technologies that enable the simultaneous screening of hundreds of individual glycan structures. Improved glycan synthesis has also enabled the development of glycan-specific antibodies, which have become more readily available. Standard ‘-omics’ technologies have been applied to the study of patterns of gene expression and levels of glycan synthetic enzymes to help identify novel clinical disorders that involve altered glycans. In terms of 3D structural analysis of glycans, NMR has been used heavily throughout the years as a means of understanding the shape of glycoconjugates in solution; molecular dynamics simulations have complemented these structural analyses by modelling glycans on protein structures. There are now many different techniques at the disposal of researchers for the analysis of glycoconjugates. Supplementary Table 2 provides a list of methodologies used for the analysis of glycoconjugates, examples of the techniques and reagents used, and referenced examples of how these methods have been applied in biomedical research.

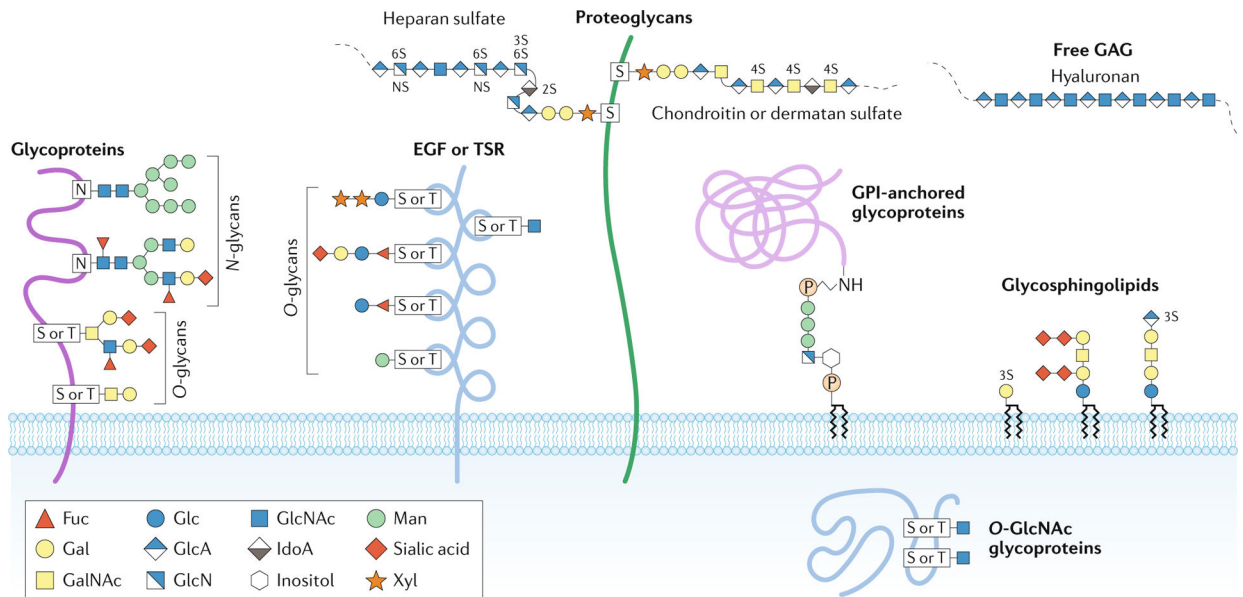


Fig. 1 | Major types of glycosylation in humans.

Glycans can be covalently attached to proteins and lipids to form glycoconjugates; glycans in these compounds are classified according to the linkage to the lipid, glycan or protein moieties. Glycoproteins consist of glycans and glycan chains linked to nitrogen and oxygen atoms of amino acid residues and are thus termed *N*-glycans and *O*-glycans, respectively. *N*-glycans consist of *N*-acetylglucosamine (GlcNAc) attached by a β 1-glycosidic linkage to the nitrogen atom of the amino group of Asn (*N*) at the consensus glycosylation motif Asn-X-Ser/Thr (in which X denotes any amino acid except for Pro). These branched and highly heterogeneous *N*-glycan structures consist of a core glycan containing two GlcNAc residues and three mannose (Man) residues. Perhaps the most diverse form of protein glycosylation is *O*-glycosylation, in which glycans attach to the oxygen atom of the hydroxyl groups of Ser (S) or Thr (T) residues. *O*-glycans can be further subclassified on the basis of the initial sugar attached to the protein and the additional sugar structures added to the initial glycan. For example, mucin-type *O*-glycosylation denotes that the initial glycan is *N*-acetylgalactosamine (GalNAc); mucin-type glycans can be further classified on the basis of the glycans attached to the initial GalNAc⁶. Other types of *O*-glycans, such as *O*-linked fucose (Fuc) and *O*-linked Man, often occur in specific proteins or protein domains, such as epidermal growth factor (EGF) repeats, thrombospondin type I repeats (TSR) or dystroglycan. *N*-glycans and *O*-glycans are often capped with negatively charged sialic acid. *O*-GlcNAc is a unique type of *O*-glycosylation that is synthesized by *O*-GlcNAc transferase; it occurs in the cytosol and nucleus. Proteoglycans represent a major class of glycoproteins that are defined by long glycosaminoglycan (GAG) chains attached to proteins through a tetrasaccharide core consisting of glucuronic acid (GlcA)–galactose (Gal)–Gal–xylose (Xyl); this carbohydrate core is attached to the hydroxyl group of Ser at Ser-Gly-X-Gly amino acid motifs. Proteoglycan GAGs can be further classified according to the number, composition and degree of sulfation of their repeating disaccharide units; common GAGs include heparan sulfate, chondroitin sulfate and dermatan sulfate. Glycosylphosphatidylinositol (GPI)-anchored glycoproteins represent another major class of

glycoconjugates. These glycoproteins are linked at the carboxyl terminus through a phosphodiester linkage to phosphoethanolamine attached to a trimannosyl-nonacetylated glucosamine (Man3-GlcN) core; the GlcN residue is linked to phosphatidylinositol, which is embedded in the cell membrane. Glycosphingolipids are a class of glycoconjugate in which glycans, such as Gal or glucose (Glc), are attached to cellular membrane lipids. Another major class of glycans is represented by GAGs that are not attached to protein cores, such as hyaluronan, which is synthesized at the plasma membrane by sequential addition of GlcA and GlcNAc. IdoA, iduronic acid. Adapted with permission from ref.²⁷⁷, Springer Nature Limited and from ref.²⁷⁸, Stanley, P. Golgi glycosylation. Cold Spring Harb. Perspect. Biol. **0033**, a005199 (2005), with permission from Cold Spring Harbor Laboratory Press.

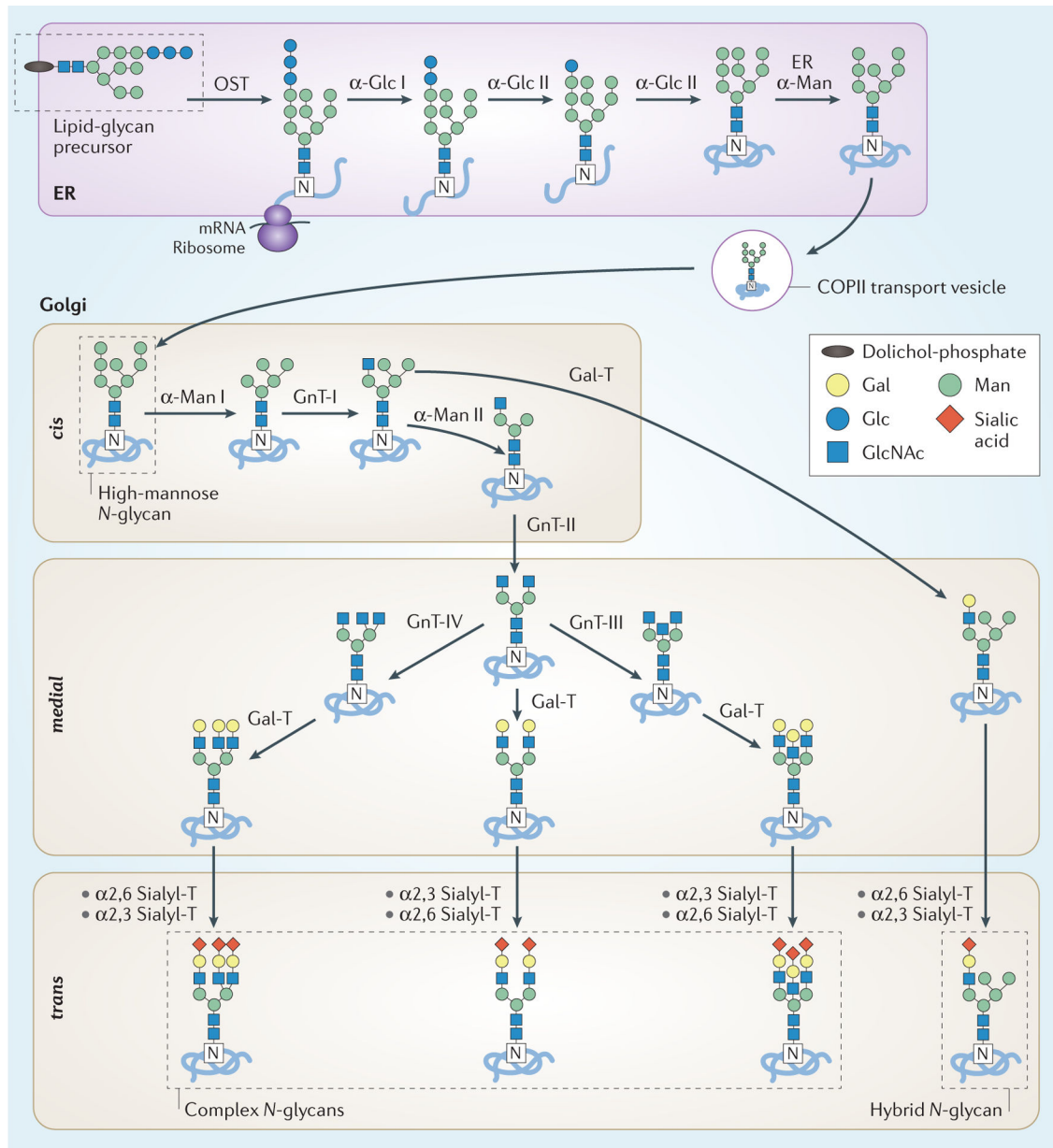


Fig. 2 | N-glycan biosynthesis in the secretory pathway.

N-glycan synthesis is initiated in the endoplasmic reticulum (ER) by the en bloc transfer of a lipid-glycan precursor (that is, glucose (Glc)³ mannose (Man)⁹ N-acetylglucosamine (GlcNAc)² bound to dolichol phosphate) to Asp by the multisubunit oligosaccharyltransferase (OST). The glucose residues are sequentially removed by two α -glucosidases (α -Glc I–II) and an initial Man residue is removed by the ER α -mannosidase (ER α -Man). After a quality-control checkpoint, the glycoprotein moves to the Golgi apparatus for additional trimming by α -mannosidase I and II (α -Man I–II) and further glycan modifications. A cis-to-trans distribution of glycosidases and transferases — GlcNAc-transferase I–IV (GnT-I–IV), β 1,4 galactosyltransferases (Gal-T), α 2,3 sialyltransferase (α 2,3 Sialyl-T) and α 2,6 sialyltransferase (α 2,6 Sialyl-T) — facilitates

further processing by these carbohydrate-modifying enzymes to create a plethora of *N*-glycoforms that often terminate with sialic acid moieties. The final site-specific *N*-glycan composition is affected by the expression levels of glycosyltransferases, the accessibility of the glycoprotein glycosylation sites and the length of time during which the glycoprotein remains in the ER and Golgi apparatus. Gal, galactose.

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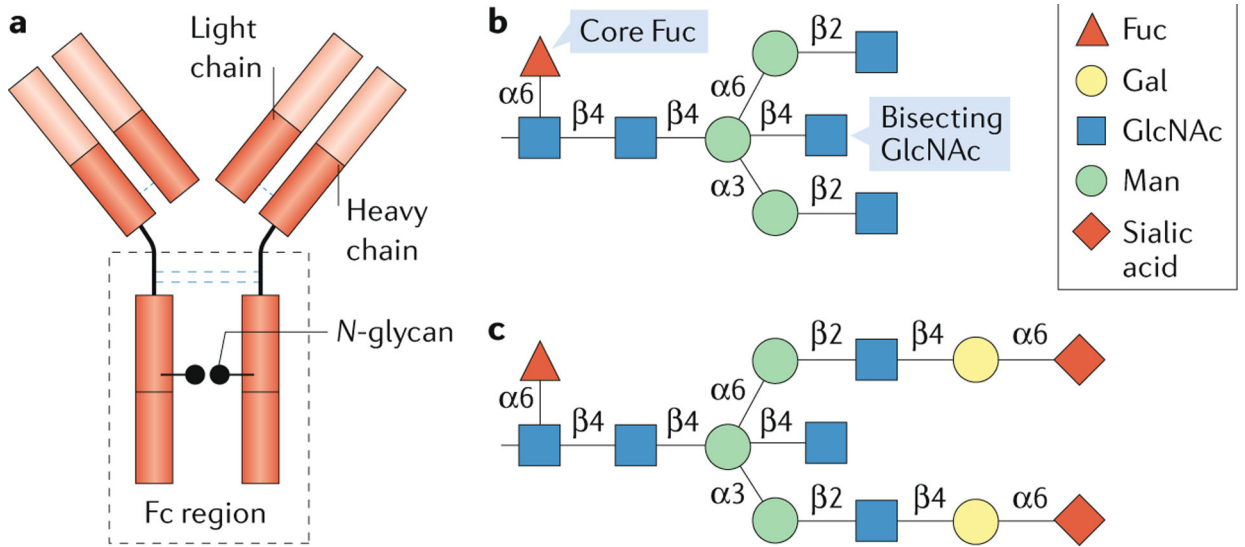


Fig. 3 | Functional impact of variable IgG Fc glycan composition.

a | Immunoglobulin G (IgG) has two heavy and two light chains, and its crystallizable fragment (Fc) region can bind to Fc γ receptors and some proteins of the complement system. The IgG Fc region contains two N-glycans, one per heavy chain, attached at Asn297; these glycans contribute to the structural integrity of the Fc region and to its interactions with Fc receptors and complement. The IgG Fc glycans in the IgG molecule are biantennary glycans with variable content of fucose (Fuc), bisecting N-acetylglucosamine (GlcNAc), galactose (Gal) and sialic acid; most IgG molecules are fucosylated. The glycan composition of IgG affects its biological activity; for example, Gal-deficient IgG glycoforms, which have been associated with chronic inflammatory diseases, can activate the lectin complement pathway. **b** | IgG glycoforms with Gal-deficient glycans are pro-inflammatory. **c** | IgG glycoforms with sialylated glycans are considered to be anti-inflammatory. Man, mannose.

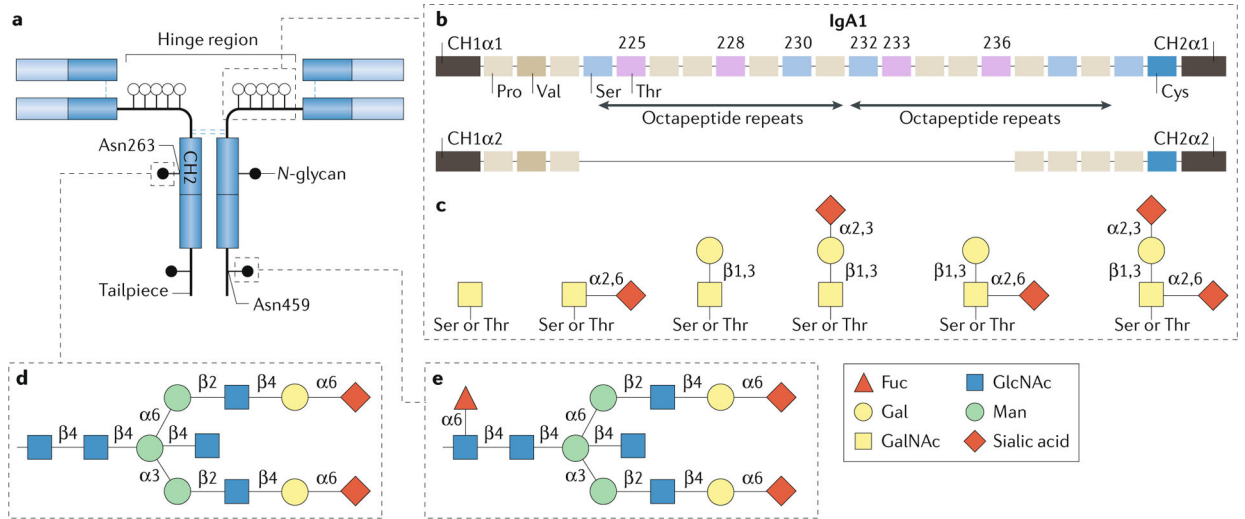


Fig. 4 | Structure and glycosylation of human IgA.

Human immunoglobulin A (IgA) occurs in two subclasses, IgA1 and IgA2. **a** | The amino acid sequence is very similar for both subclasses, but the IgA1 heavy chain contains additional amino acids in the hinge region. Each heavy chain of IgA1 also contains two *N*-glycans, one in the CH2 domain (Asn263) and one in the tailpiece (Asn459). Although human IgA2 does not contain *O*-glycans, it can have more *N*-glycans per heavy chain than IgA1. **b** | The IgA1 hinge region is composed of two octapeptide repeats, which include nine Ser and Thr residues that are potential *O*-glycosylation sites; usually 3–6 of these residues are *O*-glycosylated and the most common IgA1 glycoforms have 4–5 *O*-glycans in the hinge region. **c** | The *O*-glycan composition of normal circulating human IgA1 is variable but usually consists of a core 1 disaccharide structure with N-acetylgalactosamine (GalNAc) in β 1,3-linkage with galactose (Gal); each of these monosaccharides can be sialylated. **d** | The CH2 site of *N*-glycosylation contains digalactosylated biantennary glycans with or without a bisecting *N*-acetylglucosamine (GlcNAc), but it is not usually fucosylated. **e** | By contrast, the tailpiece *N*-glycosylation site contains fucosylated glycans. Fuc, fucose; Man, mannose.

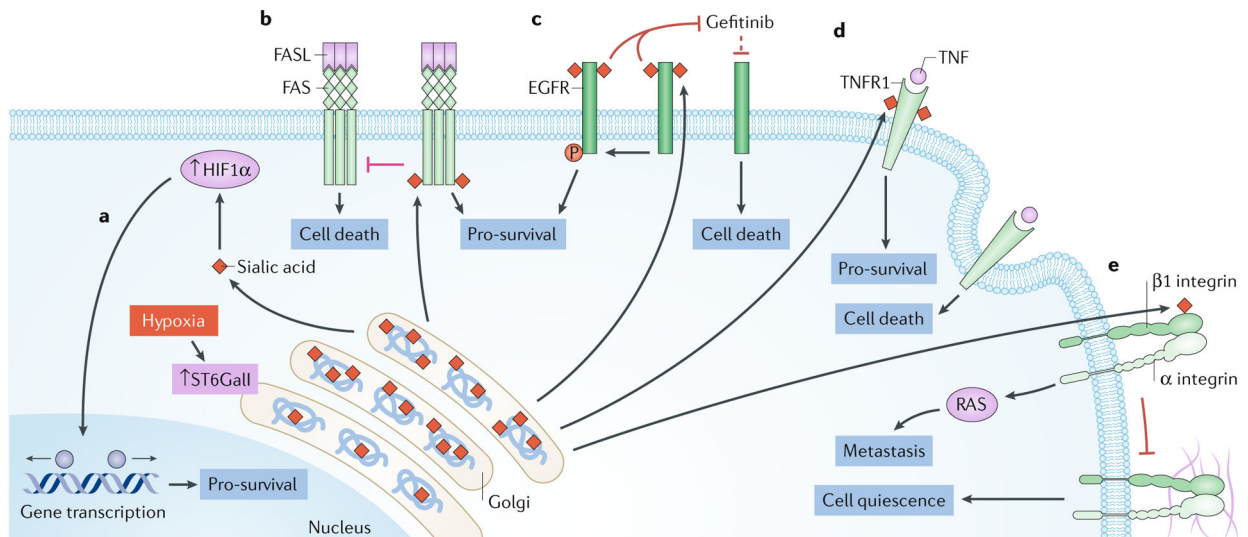


Fig. 5 | ST6galII and abnormal sialylation in cancer.

N-glycans with terminal α 2,6-sialylation are synthesized by the β -galactoside α -2,6-sialyltransferase 1 (ST6GalI). Both upregulation of ST6GalI and increase in α 2,6-sialylation are observed in many types of cancers and are associated with negative patient outcomes. In fact, α 2,6-sialylation is involved in the regulation of many key proteins that are known to contribute to cell survival and metastasis in cancer. **a** | Hypoxia increases the expression and activity of ST6GalI, leading to enhanced α 2,6-sialylation; in turn, increased sialylation upregulates HIF1 α and the expression of pro-survival HIF1 α target genes, such as growth factors and glucose transporters. **b** | The death receptor FAS, also known as CD95, is a target of ST6GalI, and its sialylation inhibits the initiation of apoptotic signalling and subsequent receptor internalization. Increased expression of ST6GalI prevents FAS ligand (FASL)-induced apoptosis through FAS. **c** | Increased expression of ST6GalI in cancer cell lines enhances the α 2,6-sialylation of epidermal growth factor receptor (EGFR), which increases its tyrosine kinase activity and the phosphorylation of its targets²⁷⁹. α 2,6-Sialylation enhances the activity of EGFR, both at baseline and after cell activation, and leads to increased activation of pro-growth and survival genes. Moreover, cells in which ST6GalI is overexpressed, leading to enhanced α 2,6-sialylation, are protected against cell death induced by the anticancer drug gefitinib, an EGFR inhibitor. **d** | In cells with low ST6GalI expression and reduced α 2,6 sialylation, prolonged activation of tumour necrosis factor (TNF) receptor 1 (TNFR1) by TNF leads to receptor internalization, caspase activation and cell death. This apoptotic cell death pathway is prevented by enhanced α 2,6-sialylation of TNFR. **e** | α 2,6-Sialylation of β 1 integrin in the Golgi apparatus by ST6GalI results in hypersialylation, which inhibits β 1 integrin binding to matrix proteins such as type I collagen and fibronectin and prevents downstream signalling. These signals maintain cell quiescence, and their disruption due to enhanced sialylation leads to increased cell motility and invasion, which promotes cancer cell metastasis.

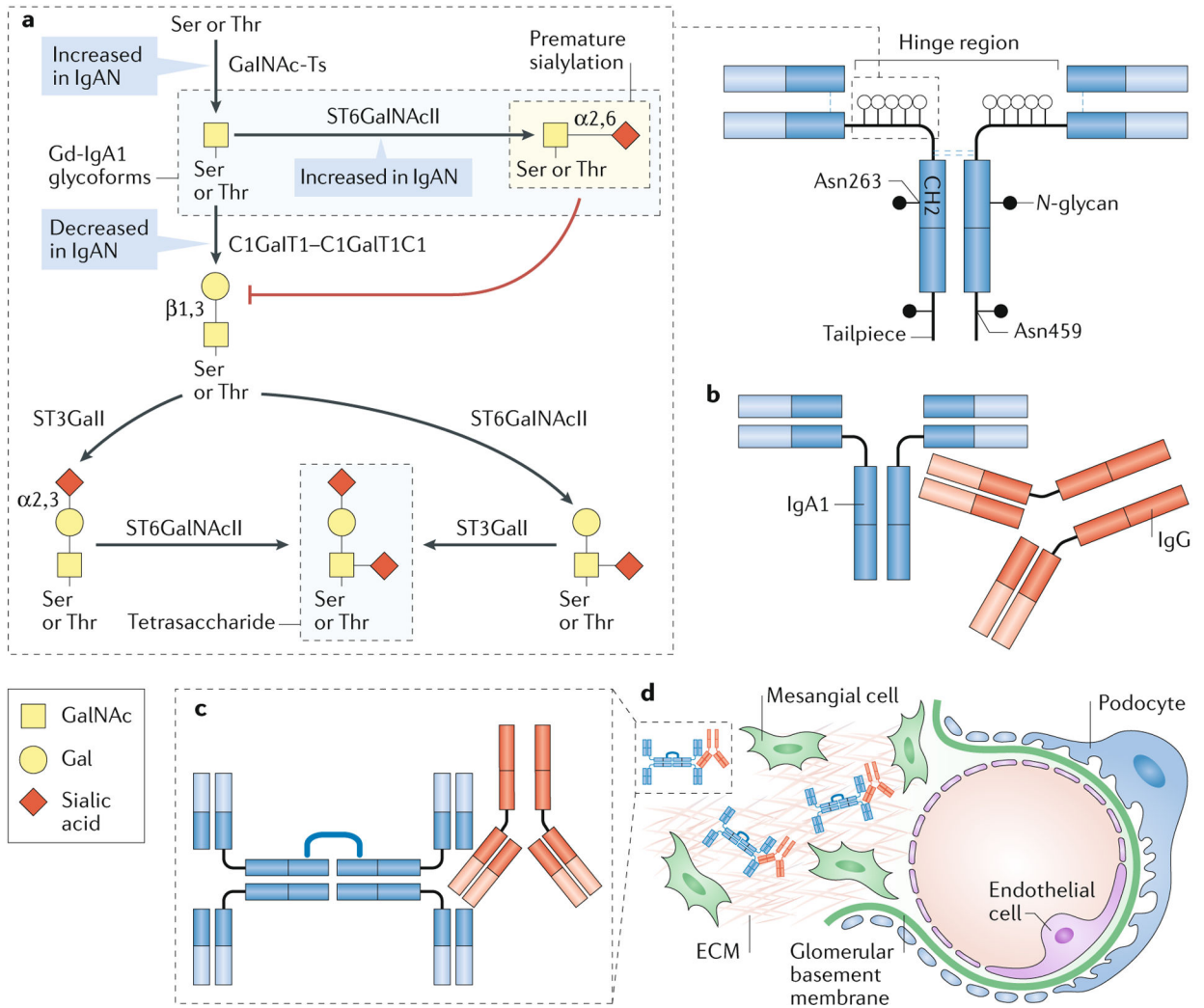


Fig. 6 | Aberrant O-glycosylation in IgA nephropathy.

Immunoglobulin A (IgA) nephropathy (IgAN) is an autoimmune disease that is thought to result from a four-hit process. **a** | Hit 1: increased production of circulating galactose (Gal)-deficient IgA1 (Gd-IgA1), in which some O-glycans do not contain Gal. The first step in the glycosylation of the hinge region of IgA1 is the addition of N-acetylgalactosamine (GalNAc) to Ser or Thr residues by a GalNAc-transferase (GalNAc-T) to form the terminal GalNAc (Tn) antigen. Premature sialylation of the terminal GalNAc by α -GalNAc α -2, 6-sialyltransferase 2 (ST6GalNAcII) can block subsequent glycosylation; however, most often GalNAc is galactosylated by the glycosyltransferase GalNAc β -galactosyltransferase 1 (C1GalT1). The chaperone C1GalT1C1 is required for appropriate expression and function of C1GalT1. After galactosylation, GalNAc, Gal or both sugars may be sialylated; ST6GalNAcII adds sialic acid to GalNAc and β -galactoside α -2,3-sialyltransferase 1 (ST3GalII) sialylates Gal; the largest glycan of circulatory IgA1 is a tetrasaccharide. In many patients with IgAN, the number of O-glycosylated residues in the hinge region of IgGA1 is also increased^{82,83}. Increased initiation of glycosylation by GalNAc-Ts, premature sialylation of GalNAc by ST6GalNAcII and decreased galactosylation by C1GalT1 might

each contribute to the formation of Gd-IgA1, the key autoantigen in IgAN. **b** | Hit 2: production of IgG autoantibodies that are specific for Gd-IgA1. **c** | Hit 3: formation of circulating Gd-IgA1–IgG immune complexes. For simplicity, IgA1 is shown as a dimer; IgA1 monomers are the main circulating molecular form, but polymeric IgA1 is the predominant molecular form of Gd-IgA1 in the circulation. **d** | Hit 4: glomerular deposition of immune complexes. Other serum proteins, such as complement, are likely to be involved in the formation of the pathogenic immune complexes that are deposited in the glomeruli, activate resident mesangial cells and cause renal injury. ECM, extracellular matrix.

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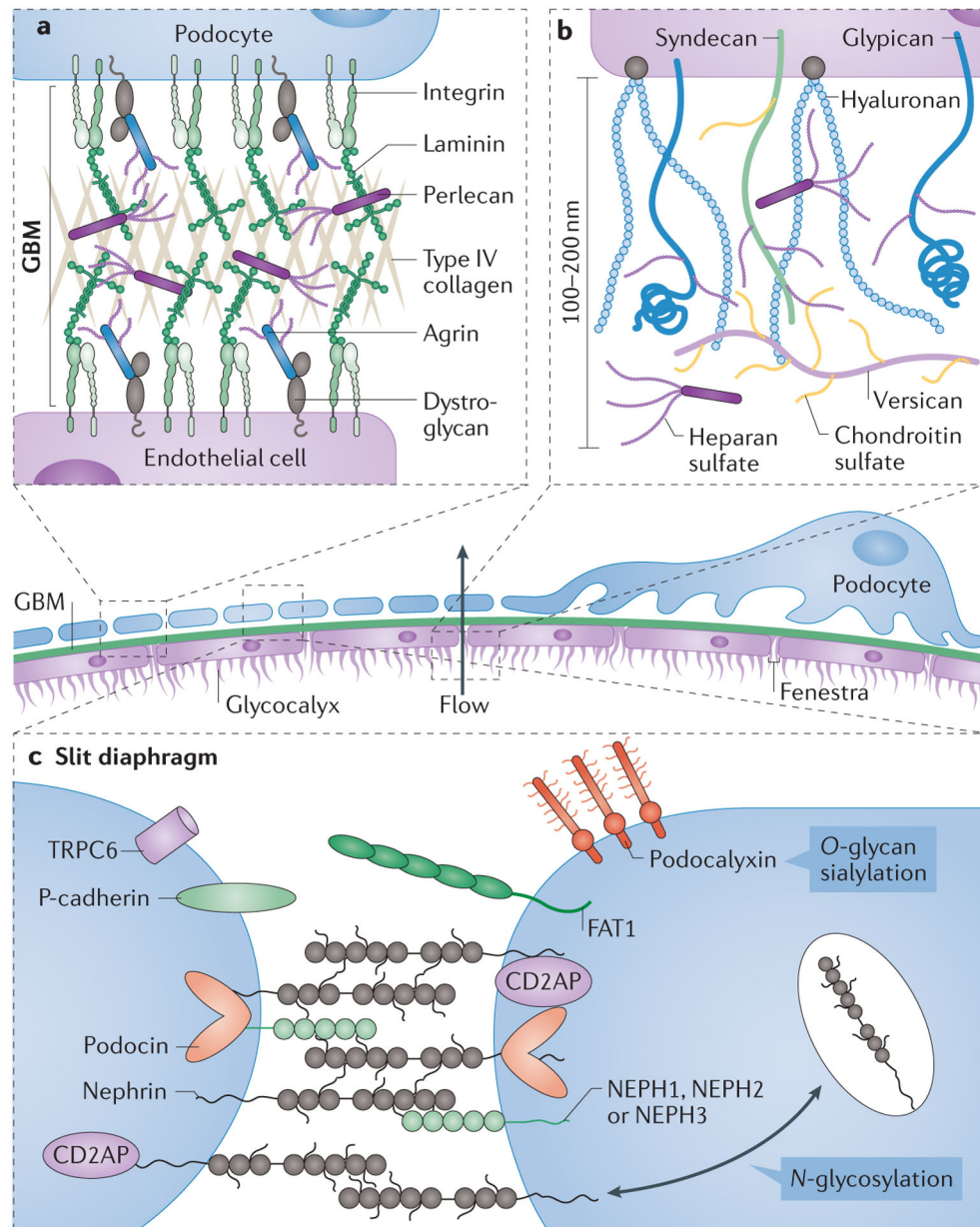


Fig. 7 | Glycoconjugates and glomerular filtration.

Glomerular filtration occurs in the glomerulus, and endothelial cells and podocytes are the principal cells involved in this process. Mesangial cells have a key role in maintaining and supporting the endothelial and podocyte glomerular filtration barrier, in part through the production of extracellular matrix proteins, and in regulating blood pressure. **a** | The glomerular basement membrane (GBM) is found at the interface between endothelial cells and podocytes; the GBM contains a network of type IV collagen, negatively charged heparan sulfate proteoglycans, laminin and several other extracellular matrix proteins²⁸⁰. Loss of proteoglycans that contain heparan sulfate, such as agrin or perlecan, occurs in some glomerular diseases, including minimal change disease and membranous nephropathy. Glycosylation of membrane-associated α -dystroglycan has an important role in the

interaction between the cells that line the GBM and the extracellular matrix through its interaction with laminin. **b** | The endothelial glycocalyx extends far from the plasma membrane of the glomerular endothelial cells, creating a physical barrier of glycoproteins, glycosaminoglycans and proteoglycans across the fenestrae. The glycan components of the glycocalyx have a key role in maintaining its mechanical and structural integrity and enabling its proper function as part of the glomerular filtration unit. Hyaluronan extends from the cell surface, whereas chondroitin sulfate and heparin sulfate are attached to extracellular matrix proteins, such as versican and perlecan, or membrane proteins such as syndecan and glypican. Together, these molecules make a dense negatively charged glycocalyx. **c** | The structure of the slit diaphragm, the key component of the glomerular molecular filter, relies heavily on the cell-surface adhesion protein nephrin; appropriate *N*-glycosylation of nephrin is critical for its surface expression and function. Sialylation of the cell-surface sialoglycoprotein podocalyxin also has a key role in podocyte morphogenesis and structural integrity. Many other cell-surface glycoproteins are involved in the formation of the slit diaphragm, including P-cadherin, podocin, nephrin-like proteins 1–3 (NEPH1–NEPH3), protocadherin fat 1 (FAT1) and transient receptor protein 6 (TRPC6). CD2-associated protein (CD2AP) is an adaptor molecule that can bind to the cytoplasmic domain of nephrin.

Table 1 |

Selected examples of congenital disorders of glycosylation

CDG ^a or syndrome	Defective gene and protein	Affected pathway	Phenotypic outcome
ALG1-CDG	<i>ALG1</i> , β 1,4-mannosyltransferase	<i>N</i> -glycan biosynthesis; ALG1 catalyses the first mannosylation step in the biosynthesis of the lipid-linked precursor oligosaccharide for <i>N</i> -glycosylation	Intellectual disability, hypotonia, seizures, microcephaly, infections and early death
B4GALT1-CDG	<i>B4GALT1</i> , β 1,4-galactosyltransferase 1	Two alternative transcripts: one encodes a <i>trans</i> -Golgi-resident protein involved in glycoconjugate biosynthesis whereas the second transcript encodes a protein that forms soluble lactose synthase	Intellectual disability, developmental delay, hypotonia, macrocephaly, Dandy-Walker malformation, coagulopathy and myopathy
COG7-CDG	<i>COG7</i> , conserved oligomeric Golgi complex subunit 7	Retrograde transport from the Golgi complex to the endoplasmic reticulum; involved in multiple glycosylation pathways	Hypotonia, microcephaly, growth retardation, adducted thumbs, failure to thrive, cardiac anomalies, wrinkled skin and early death
MPI-CDG	<i>MPI</i> , mannose-6-phosphate isomerase	Conversion of fructose-6-phosphate and mannose-6-phosphate; involved in multiple glycosylation pathways	Hepatic fibrosis, coagulopathy, hypoglycaemia, protein-losing enteropathy and vomiting; no neurological symptoms
NGLY1-CDG	<i>NGLY1</i> , PNGase	NGLY1 cleaves the β -aspartyl-glucosamine (GlcNAc) of the glycan and the amide side chain of Asn, converting Asn to Asp. This process assists in proteasome-mediated degradation of misfolded glycoproteins	Intellectual disability, developmental delay, seizures, abnormal liver function and hypolactrima or alacrima
PMM2-CDG	<i>PMM2</i> , phosphomannomutase 2	Conversion of mannose-6-phosphate to mannose-1-phosphate; involved in multiple glycosylation pathways	Intellectual disability, hypotonia, seizures, strabismus, cerebellar hypoplasia, failure to thrive and cardiomyopathy; 20% lethality in the first 5 years of life
SRD5A3-CDG	<i>SRD5A3</i> , polyprenol reductase	SDR5A3 enzyme converts polyprenol into dolichol, a key step in the synthesis of dolichol-linked monosaccharide and the oligosaccharide precursors for <i>N</i> -glycosylation	Intellectual disability, hypotonia, eye and brain malformations, nystagmus, hepatic dysfunction, coagulopathy and ichthyosis
CHIME syndrome	<i>PIGL</i> , GlcNAc-PI de- <i>N</i> -acetylase	PIGL catalyses the second step of GPI biosynthesis, de- <i>N</i> -acetylation of GlcNAc-PI	Coloboma, congenital heart disease, ichthyosiform dermatosis, mental retardation and ear anomalies syndrome (CHIME)
GENE myopathy	<i>GENE</i> ; bifunctional UDP-GlcNAc-2-epimerase/MannNac kinase	Biosynthesis of sialic acid	Proximal and distal muscle weakness, wasting of the upper and lower limbs and selective sparing of the quadriceps
Walker-Warburg syndrome	Various genes, including <i>POMT1</i> and <i>POMT2</i> that encode two subunits of <i>O</i> -mannosyltransferase	Malfuction of various enzymes that modify α -dystroglycan	Defects in muscle, brain and eye development and elevated serum creatine kinase

CDG, congenital disorder of glycosylation; GlcNAc, *N*-acetylglucosamine; GPI, glycosylphosphatidylinositol; ManNAc, *N*-acetylmannosamine; PI, phosphatidylinositol.

^aCurrent recommended nomenclature for CDGs is used, XX-CDG, in which XX defines the specific affected gene.

Table 2 |

glycan-based therapeutics currently available or under development

Disease	Treatment	Outcome	Refs
<i>Glycan-supplementation therapeutics</i>			
PGM1-CDG	Oral D-galactose supplementation	Improved liver function and normalized glycosylation markers and UDP-galactose levels	38
MPL-CDG	Oral mannose supplementation	Significant reduction in disease activity	38
CAD-CDG	Oral uridine supplementation	Significant reduction in disease activity	38
<i>Vaccine therapeutics</i>			
Non-metastatic castration-resistant prostate cancer	Recombinant Tn-rmMUC1 peptide vaccine	PSA doubling time improved in phase I and phase II clinical trials	281
Breast cancer	MUC1 glycopeptide vaccine	Clinical trials in progress to evaluate immunogenicity and anticancer effects	125
HIV-1	Increased high-mannose glycans of gp120 antigen	Enhanced binding of broadly neutralizing antibodies to HIV gp120	239
<i>Streptococcus pneumoniae</i>	Bacterial strain-specific polysaccharide vaccine	Increased production and affinity of antigen-specific IgG in mouse model	47
<i>Therapeutic antibodies</i>			
Adult T cell lymphoma	Defucosylated anti-CCR4 antibody	Overall response rate of 50%	249
Rheumatoid arthritis	Aglycosylated anti-CD4 antibody	Modest reduction in disease activity	257
Type 1 diabetes	Aglycosylated anti-CD3 antibody	Moderate efficacy to stabilize insulin secretion levels over time	255

CCR4, CC-chemokine receptor 4; IgG, immunoglobulin G; PSA, prostate-specific antigen; rmMUC1, recombinant mucin 1; Tn, terminal *N*-acetylgalactosamine.