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Potential of glycosylation research in graft versus host disease after allogeneic hematopoietic stem cell transplantation

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Abstract

Background—Glycans, complex oligosaccharides, are directly involved in almost every biological process, have a fundamental role in the immune system and are probably involved in nearly every human disease. However, glycosylation has been greatly ignored in the area of allogeneic hematopoietic stem cell transplantation (alloHSCT) and graft versus host disease (GVHD). Both acute and chronic GVHD are multisystemic debilitating immunological disturbances arising after alloHSCT.

Scope of Review—In this paper we review the glycosylation research already done in the field of alloHSCT and GVHD, and evaluate further potential of glycan analysis in GVHD by looking into resembling inflammatory and autoimmune conditions.

Major Conclusions—Glycan research could bring significant improvement in alloHSCT procedure with reduction in following complications, such as GVHD. Identifying glycan patterns that induce self-tolerance and the ones that cause the auto- and allo-immune response could lead to innovative and tissue specific immunomodulative therapy instead of the current immunosuppressive treatment, enabling preservation of the graft-versus-tumor effect. Moreover,

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improved glycan pattern analyses could offer a more complete assessment and greatly needed dynamic biomarkers for GVHD.

General Significance—This review is written with a goal to encourage glycan research in the field of alloHSCT and GVHD as a perspective tool leading to improved engraftment, discovery of much needed biomarkers for GVHD, enabling an appropriate therapy and improved monitoring of therapeutic response.

Keywords

glycan; glycosylation; graft versus host disease; hematopoietic stem cell transplantation

1. Introduction

Glycans – complex oligosaccharides – are a major component of the cell: they exist on the surface of every cell and are a part of almost all membranes (attached to lipids and proteins) and secreted proteins [1]. Numerous cells, protein receptors and soluble mediators of the immune system, such as class I and class II major histocompatibility complex proteins, T and B cell receptors, chemokine and cytokine receptors, and antibodies, contain significant amounts of covalently attached glycans [2]. Glycans play important roles in major biological events (such as cell–cell interaction, protein folding and receptor binding) and are directly involved in almost every biological process [1]. They have already been associated to a number of inflammatory conditions [3], autoimmune diseases and hematological cancers [4,5]. In addition, it is suggested that glycans have a major role in nearly every human disease [1,6,7].

Recent advances in the field of glycan pattern analyses hold great promise for understanding various diseases mechanisms and for biomarkers research. In this review, we present current knowledge of glycosylation characteristics and its possible role and research potential in the field of graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (alloHSCT).

2. Glycans: biology and analysis

Glycans are non-linear branched oligosaccharides, structurally extremely complex. Because of their molecular complexity, the absence of a direct genetic template and methodological difficulties, glycan research lagged behind genomics and proteomics. Recent studies showed that glycans are a product of both genetic and environmental factors [8], and that glycosylation is a tightly regulated process where different glycan attachments are of great biochemical importance [9,10].

Ten commonly found monosaccharide building blocks compose numerous diverse combinations of human oligosaccharides which vary in type, number, order and spatial relation of monosaccharide units. Number of possible variations grows even further since hydroxyl groups of different monosaccharides can become subject to phosphorylation, sulfation, methylation, O-acetylation, or fatty acylation.

Glycoproteins are glycoconjugates in which glycans are covalently linked to a polypeptide backbone, usually via *N*- or *O*-linkages. Addition of complex oligosaccharides to polypeptide backbone is the most abundant and the most structurally diverse posttranslational modification of proteins. It greatly affects protein conformation and leads to changes in the protein behavior affecting its biological functions.

Recognition of glycans and transfer of information consisted in the sugar moiety is being done via glycan-binding proteins, which can be sorted in two classes: lectins and glycosaminoglycan-binding proteins. Lectins recognize *N*-glycans, *O*-glycans and glycosphingo-lipids, while glycosaminoglycan-binding proteins tend to bind different types of sulfated glycosaminoglycans [11]. Review of basic glycobiology terminology is given in Table 1.

Glycosylation analysis can be performed on whole serum *N*-glycan profile as well as on specific glycoproteins, such as immunoglobulin (Ig) G and A. One of the most analyzed glycoproteins is IgG, the most abundant class of antibody in the human plasma (around three quarter of serum Igs) [12]. IgG carries *N*-linked glycans at constant domain 2 (CH2, Asn 297) of its Fc region, most of which are of a complex type with a biantennary heptameric core (three mannose and four *N*-acetyl-glucosamine residues) and possible additions of *N*-acetylglucosamine, fucose, galactose and sialic acid residues (Figure 1.A). The attached sugar shows great variability with more than 30 identified different IgG glycosylation variants for any of the four different human IgG subclasses [13]. The IgG glycome composition is rather stable in healthy individuals, but inter-individual differences are very large [14,15], with both genetic, epigenetic and environmental factors contributing to these differences [4]. Despite significant heritability of the steady-state composition [16], IgG glycome composition can change rapidly in the state of proinflammatory response [17] (Figure 1.B.). The composition of the IgG *N*-glycan affects the protein conformation and subsequently its ability to bind to the FcγRs which can modulate ADCC [18,19], complement activation [20] and other immune responses. Minute changes in the IgG N-glycan composition influence its FcR affinity. For example, lack of core fucose increases affinity for FcγRIIIa receptor leading to an improved effector function [21]. The addition of terminal sialic acid changes the conformation of the protein and initiates an anti-inflammatory cascade by binding to an alternative class of receptors [22]. Differences in abundances of IgG glycan traits in patients with different autoimmune diseases sets them as a leading candidate for new biomarkers for various autoimmune diseases [23,24] (Figure 1.C.).

3. Glycans in alloHSCT

Allogeneic HSCT is a potentially lifesaving procedure for a variety of hematological malignant and non-malignant disorders. Although there are areas of alloHSCT that could benefit from better understanding of glycosylation mechanisms involved, glycosylation analysis has been greatly ignored in alloHSCT.

3.1. Engraftment and glycan research

A successful alloHSCT depends upon the ability of infused hematopoietic stem cells (HSCs) to home from blood to the bone marrow (BM) cavity to reestablish productive hematopoiesis. Such homing is a nonrandom process regulated by adhesive interactions between HSCs and BM endothelium. Marrow endothelial cells constitutively express E-selectin [25], a member of selectin family of adhesion molecules that binds to sialofucosylated glycans expressed on glycoproteins or glycolipids of circulating cells. Engagement of E-selectin promotes homing of circulating HSCs to BM [26]. E-selectin on the BM vasculature is also known to directly induce HSC proliferation at the expense of HSC self renewal [27].

Integral membrane glycoprotein hematopoietic cell E-/L-selectin ligand (HCELL), a specialized glycoform of CD44, has been identified as the predominant E-selectin ligand on circulating human HSC [28]. Glycans of BM hematopoietic stem and progenitor cells are suggested to be remodeled by a remotely produced glycosyltransferase which mediates the formation of cell surface α2,6-linked sialic acids important for cell interaction with other cells and its surroundings [29]. Glycosylation manipulation of naïve CD44 on a cell unable to achieve tissue-specific migration could be custom modified into HCELL glycoform without affecting cell viability or native phenotype. The method is called ‘glycosyltransferase-programmed stereosubstitution’ [30] and could potentially improve delivery of HSC into BM with a purpose of a more successful reconstitution of hematopoiesis. Glycoengineering is also considered as a solution to poorer engraftment results of cord blood transplantation, often limited by a low number of HSC contained in the graft. *Ex vivo* fucosylation of cord blood HSC using recombinant human fucosyltransferases is currently being tested as a promising method to improve the rate and magnitude of engraftment [31].

Glycans could also help to clarify some of the complex interactions between donor’s and host’s immunological systems after alloHSCT, including one of the more severe consequences–graft versus host disease. This post-transplant complication, occurrs in two distinct, clinically well characterized forms: acute and chronic.

3.2. Acute graft-versus-host disease (aGVHD)

There are two subcategories of aGVHD: (1) classic (presenting itself with typical aGVHD symptoms within 100 days of alloHSCT or donor lymphocyte infusion (DLI)) and (2) persistent, recurrent, or late-onset aGVHD with features of classic aGVHD occurring beyond 100 days after alloHSCT or DLI in a patient not meeting criteria for the diagnosis of chronic GVHD (cGVHD)[32,33]. Acute GVHD affects skin, gastrointestinal system and liver. Typical presentations include erythema and/or maculopapular rash on the palms and soles, secretory diarrhoea and cholestatic liver disease [34]. Incidence reaches up to 50% of patients receiving HLA-matched transplants from a related donor and up to 70% of those receiving transplants from an unrelated donor [35].

Studies emphasize the role of innate immunity in the initiation of aGVHD. Total body irradiation and chemotheraphy cause an injury of gastrointestinal mucosa allowing the

transit of commensal bacteria from the lumen. Host's pattern recognition receptors recognize PAMPs and DAMPs, glycoproteins also known as alarmins, following the conditioning regimen and activate the innate immune system. This reaction is supported by inflammatory cytokines secreted by the damaged tissue which leads to recruitment of effector cells and enhances the recognition of host alloantigens by donor-derived T-cells [36,37]. The described process initiates a complex cascade of tissue destruction creating a pro-inflammatory milieu, which amplifies and perpetuates aGVHD. Since aGVHD mostly manifests itself in three locations – skin, liver and gut – it is obvious that immune cells are being navigated towards those organs. Glycans are crucial in cell communication and recognition, and highly involved in trafficking of immune cells. Hence, manipulation of immune cell trafficking and regional immunity seems like a promising area of research. Glycans are also well known as markers of acute systemic inflammation and could hopefully aid in clarifying GVHD pathogenesis. For example, it has been demonstrated that in a whole-body inflammatory reaction (such as a cardiovascular surgery) certain glycan groups of plasma proteins show rapid and uniform increase. Results from that research indicated that it is possible to predict the severity of acute inflammatory response and identify individuals with a higher mortality risk prior to an invasive procedure based on the level of fucosylation of IgG *N*-glycans [38]. Changes of serum glycans have also been described in other inflammatory conditions, such as sepsis and acute pancreatitis, early in the acute phase response [38,39]. Since glycan profiles in healthy serum are more or less constant, it is suggested they could have a valuable prognostic potential [17], which might also be exploitable in GVHD.

Another candidate in demystifying GVHD could be galectin-9 (Gal-9). This molecule is a member of the galectin family of carbohydrate-binding proteins (lectins), and it is often associated with modulation and homeostasis of T cells. Elevated expression of endogenous Gal-9 was found in the process of rejection of allografted solid organs, correlating with the progression of the process [40]. It has been found to promote differentiation of naïve T cells into regulatory T cells [41]. It also represses differentiation into T-helper 17 cells and induces apoptosis in mature CD4+ T cells, and CD8+ [42]. Recombinant Gal-9 has already been tested on a mouse model of aGVHD with promising results [43].

3.3. Chronic graft-versus-host disease (cGVHD)

Chronic GVHD is the major late complication following alloHSCT, associated with increased mortality, impaired physical and functional status and decreased quality of life [44]. It is a systemic alloimmune and autoimmune disease characterized by immune deregulation, immunodeficiency, and development of signs and symptoms of various autoimmune disorders targeting multiple organ systems (oral, digestive and genital mucosa, glandular tissue, skin, eyes, lungs, liver, and joints). Reported incidence rates of cGVHD range from 30–70% [45,46] according to recipient age, donor type, stem cell source and use of posttransplantation DLIs.

In 2005, the National Institutes of Health (NIH), USA, convened a cGVHD Consensus Conference defining the new conceptual understanding of cGVHD, with the new scoring system based on number of organs involved, severity, and functional disability [32,47–51].

In June 2014, the second cGVHD consensus conference was held at the National Cancer Institute, NIH, USA. Updated recommendations about cGVHD diagnosis, staging, histopathology, biomarkers, response criteria, design of clinical trials, ancillary and supportive care resulted from that second cGVHD conference [33,52–56].

In spite of huge effort and progress in cGVHD after two NIH consensus meetings, there are still many unresolved questions regarding understanding of the pathophysiology and predicting occurrence of cGVHD, improving diagnosis and staging, measuring short-term responses to treatment and predicting long-term clinical benefit. Moreover, there is still no USA Food and Drug Administration or European Medicines Agency approved agent for cGVHD treatment or prevention, and the first line treatment with steroids has 50% failure rate with significant steroid toxicity. There is no standard second and subsequent line of therapy, and preventive and preemptive strategies to decrease incidence and severity of cGVHD are not standardized.

As we know today, cGVHD is a result of complex series of immune interactions that occur as the donor immune system develops in antigenically disparate recipient environment. Although it has been demonstrated that donor T cells transferred along with the allograft are the primary immunocompetent cells that induce GVHD [57], increased attention in this systemic immunological disturbance is being directed towards additional cell populations [58], especially B cells [59–62]. It is known that B-cell reconstitution in patients with cGVHD is delayed, and these patients have elevated plasma B-cell activating factor (BAFF)/naïve B-cell ratio [63,64]. The unique post-alloHSCT surrounding with high levels of BAFF is known to promote the differentiation and survival of allo- and auto-reactive B cells [65]. Successful treatment with high-dose prednisone was associated with reduced BAFF levels in patients with active cGVHD, whereas lower concentration of BAFF were measured in patients who never developed cGVHD [66]. Involvement of B cells would also explain the partial success of rituximab (anti-CD20 monoclonal antibody), drug used both in prophylaxis [67,68] and as a second line of steroid refractory cGVHD [69]. New insights into the rituximab mechanism of action suggest that the partial response to the drug could be due to the polymorphism of FcγR. It has been reported that follicular lymphoma patients with higher affinity allelic variants of receptor FcγRIIIa have a better response than patients with low-affinity polymorphisms [70], which could be applicable to cGVHD. One of the possible resolutions of the incomplete response to rituximab may be Fc-glycoengineering in order to enhance ADCC either by reducing *N*-glycan core fucosylation or incorporation of bisecting sugar residue [71]. Although a recent study described that patients with long-lasting cGVHD have a trend towards higher incidence of allo- and auto-antibodies (AAbs) [72], it has not been linked to the severity or activity of neither the disease, nor it has been elucidated whether these are pathogenic or represent a consequence of disturbed B-cell homeostasis.

Distortion of B cell homeostasis and production of AAbs common for cGVHD can be compared to autoimmune diseases, where the pathogenic role of AAbs has been confirmed [73–77]. Clinical observations also support the autoimmune nature of the disease, since cGVHD is well known for mimicking autoimmune disorders such as Sjögren syndrome

(SS), systemic lupus erythematosus (SLE), myositis, immune cytopenia, and others (Table 2.).

4. Glycosylation patterns in various autoimmune diseases resembling cGVHD

Autoimmune-like features of cGVHD have already been reviewed on several occasions [78,79] and here are described in the light of glycosylation research potential.

Some of the more common symptoms of cGVHD are xerostomia and dry eyes, resembling SS, an autoimmune disease causing a functional impairment of salivary and lacrimal glands. It is considered that B cells are over-stimulated and produce excessive amounts of Igs and various AAbs in SS [74]. Elevated levels of asialylated IgG were detected in SS patients [80], and recent publications suggested altered glycosylation of salivary mucins which reduces lubricating properties and quality of the saliva [81]. This mechanism could explain the sensation of dry mouth even if the saliva volume and the glands remain intact and might be tested in cGVHD.

Development of AAbs and breakdown in B cell tolerance is characteristic of SLE [82], and glycosylation is also disturbed in this comprehensive immunological disorder. Increased glycan moiety in α2-macroglobulin and the significantly higher content of galactose in the glycan of the same protein was reported [83]. Recent extensive study of three independent cohorts of SLE patients also observed significant changes in glycome composition, which correlate to the symptom severity [24]. Changes of IgG sialylation have also been suggested as a novel biomarker for distinguishing patients with SLE from patients with other autoimmune diseases [84].

Gastrointestinal GVHD resembles inflammatory bowel disease (IBD), another autoimmune disorder. Inflammation in IBD is believed to be triggered by an aberrant immune response to gut microbiota in genetically susceptible individuals. Intestinal mucus of IBD patients shows decreased glycosylation which gives rise to increased bacterial contact with the epithelium and potentially triggers inflammation [85]. A recent research indicated a significantly increased inflammatory potential of IgG in IBD due to changes in its glycosylation. These changes are considered to contribute to the disease pathogenesis, and have been suggested as the biomarker of the disease onset and severity [23,86]. Dysregulation of T cell receptor *N*-glycosylation is also believed to be a part of the mechanism of ulcerative colitis, a type of IBD. Patients with severe degree of the disease showed a defect in *N*-glycan branching in T cell receptor [87], which might be tested in GVHD patients.

Autoimmune hematological diseases are frequently reported to occur following HSCT [79,88]. Thrombocytopenia in cGVHD could be autoimmune mediated but may as well have multifactorial etiology, and it is one of the risk factors for poorer survival in cGVHD patients [89–91]. AAbs which are likely to contribute to the increased rate of platelet destruction may develop, as in immune thrombocytopenia (ITP) [33]. It has been shown that IgG *N*-glycans are involved in the pathophysiology of ITP [92]. *In vitro* experiments demonstrated decreased phagocytic activity of monocytes mediated by deglycosylated AAbs compared to

native AAbs from ITP patiens. Cleavage of carbohydrates also interfered with Fc-mediated phagocytosis and complement activation and prolonged platelet survival *in vivo*.

Neurological manifestations, such as myasthenia gravis, myositis and immune-mediated neuropathies [93], have also been described as a part of the cGVHD clinical picture. Clinicians are often challenged in discriminating the damage caused by cGVHD from drug-induced toxicities, long-term immunosuppression or opportunistic infections and glycans could represent a tool to resolve that dilemma. Myasthenia gravis is an antibody-mediated autoimmune disorder of the neuromuscular junction. *N*-glycosylation analysis of total IgG showed lower levels of IgG2 galactosylation in myasthenia gravis patients compared to controls, while there were no notable differences in the IgG core-fucosylation and the overall degree of sialylation remained similar [94]. Myositis patients had overall elevated amounts of IgG glycoforms lacking terminal galactose [95].

5. Conclusions and future considerations

Recent advances in technology of glycan analysis give hope of promising future research in alloHSCT and GVHD to better understand consequences of merging two immunological systems. Identifying glycan patterns that induce self-tolerance and the ones that cause the auto- and allo-immune response could lead to improved alloHSCT procedure. Some of the potential therapy ideas include remodeling of glycosylated proteins *in vivo*. Possibility of HSC manipulation and successful control of the engraftment process could result in further reducement of conditioning intensity, improved delivery of HSCs to their niche, cut down in number of graft rejections and enable transplatations with grafts limited with low HSC number, such is cord blood. This kind of innovative and tissue specific immunomodulative therapy could help us move past current immunosuppressive treatment with its toxic side-effects, enabling preservation of the graft-versus-tumor effect. This technology could also enable us to interfere with lymphocyte homing to the site of inflammation or restructure glycoforms contributing to the rise of acute and/or chronic GHVD [96]. In addition to that, neglected glycans could possibly represent candidate biomarkers for GVHD, much needed to diagnose and monitor the disease, prognose its course and outcome, and characterize its activity. For example, anti-glycan antibodies profiling is a new and promising tool and such antibodies have already been suggested as biomarkers for multiple sclerosis and IBD [97]. Systematic screening of blood could lead to a timely discovery of glycoforms indicative of developing acute or chronic GVHD or biomarkers specific for the organ system endangered by GVHD - enabling an appropriate therapy and monitoring of therapeutic response. To conclude, it is likely to expect that future research of glycans in the field of alloHSCT and GVHD would improve outcome of those patients.

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Highlights

- Glycans are involved in almost every biological process
- Glycans are associated with immunological disturbances and autoimmune diseases
- GVHD is alloimmune and autoimmune disorder following alloHSCT
- Glycans have a great research potential in clarifying events after alloHSCT and GVHD
- Glycan research could lead to improved therapy and discovery of biomarkers

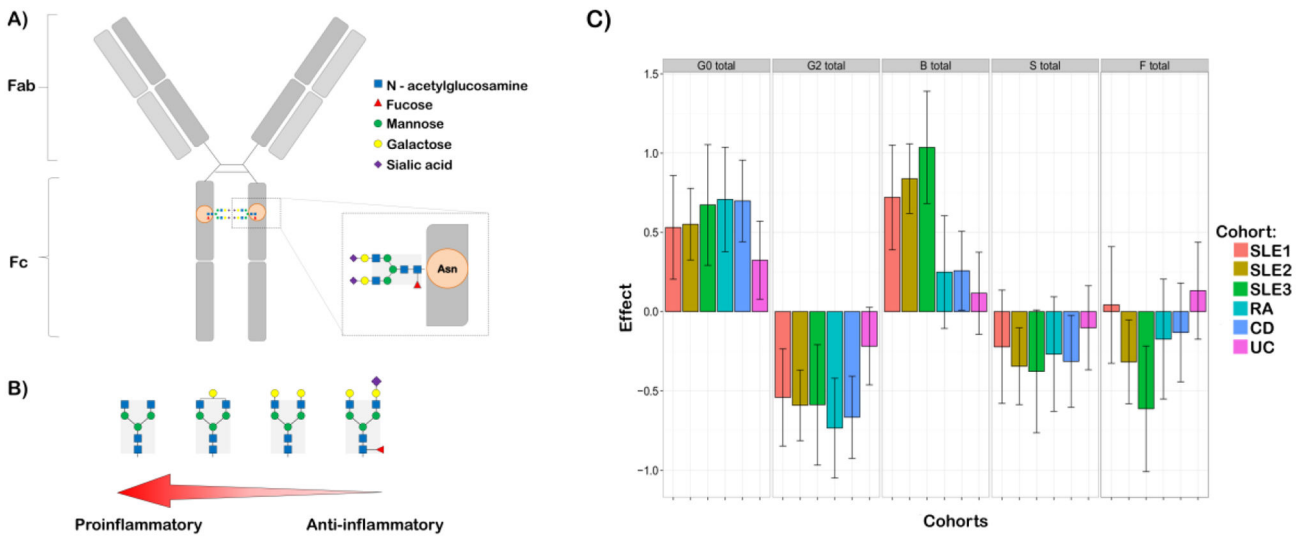

Figure 1.

A) Three mannose and four *N*-acetyl-glucosamine residues comprise a biantennary heptameric core (highlighted in grey) of IgG glycans. Possible additions of *N*-acetylglucosamine, fucose, galactose and sialic acid residues make up to 30 possible glycosylation variants. The sugar moiety is attached to the Asn 297 of constant Fc region of IgG. B) Minute changes in structure of the IgG *N*-glycan modulate the inflammatory activity of IgG (depiction according to Maverakis et al., 2015(7)). C) Differences in abundances of IgG glycan traits in patients with different autoimmune diseases (depiction according to data published in I. Trbojevic Akmacic et al., 2015 and F. Vuckovic et al., 2015(23,24)).
G0 total – total agalactosylated glycans; G2 total – total digalactosylated glycans; B total – total glycans with bisecting GlcNAc; S total – total sialylated glycans; F total – total fucosylated glycans; SLE1-3 – Latin America, Trinidad and Chinese cohorts of patients with systemic lupus erythematosus; RA – Chinese cohort of patients with rheumatoid arthritis; CD – UK cohort of patients with Chron's disease; UC – UK cohort of patients with ulcerative colitis.

Table 1

Glossary and review of glycobiology terminology mentioned in the article (according to Varki et al. [11]).

ADCC	Antibody dependent cell-mediated cytotoxicity.
Alarmins	Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), glycoproteins also known as alarmins.
DAMPs	See *Alarmins*.
Fab	The fragment antigen binding region, see *Immunoglobulin*.
Fc	The fragment crystallisable region, see *Immunoglobulin*.
FcR	Fc receptor, see *Immunoglobulin*.
Galectin	S-type (sulphydryl-dependent) β-galactoside-binding lectins, usually occurring in a soluble form, expressed by a wide variety of animal cell types and distinguishable by the amino acid sequence of their carbohydrate-recognition domains.
Glycan	A generic term for any sugar or assembly of sugars, in free form or attached to another molecule, used interchangeably with saccharide or carbohydrate.
Glycan-binding proteins	Proteins that recognize and bind to specific glycans and mediate their biological function. Sorted in two classes: lectins and glycosaminoglycan-binding protein.
Glycoconjugate	A molecule in which one or more glycan units are covalently linked to a noncarbohydrate entity.
Glycoform	Different molecular forms of a glycoprotein, resulting from variable glycan structure and/or glycan attachment site occupancy.
Glycoprotein	A protein with one or more covalently bound glycans.
Glycosaminoglycans	Polysaccharide side chains of proteoglycans or free complex polysaccharides composed of linear disaccharide repeating units, each composed of a hexosamine and a hexose or a hexuronic acid.
Glycosylation	The enzyme-catalyzed covalent attachment of a carbohydrate to a polypeptide, lipid, polynucleotide, carbohydrate, or other organic compound, generally catalyzed by glycosyltransferases, utilizing specific sugar nucleotide donor substrates.
Glycosyltransferase	Enzyme that catalyzes transfer of a sugar from a sugar nucleotide donor to a substrate.
Immunoglobulin (Ig)	Part of the adaptive immune system, produced by B cells and plasma cells. It consists of two heavy and two light chains. Five types of heavy chains (γ, α, μ, δ and ε) exist in mammals and the type of the heavy chain defines the class of the Ig molecule. The ‘arms’ of the Ig consisting of both the heavy and the light chains, contain the Fab region that recognizes and binds to the antigen. The Fc region binds to the corresponding FcR of immune cells and participates in the regulation of their activity.
Lectin	A protein that specifically recognizes and binds to glycans without catalyzing a modification of the glycan.
Monosaccharide	Carbohydrate that cannot be hydrolyzed into a simpler carbohydrate. It is the building block of oligosaccharides and polysaccharides. Commonly found monosaccharide building blocks: D-glucose (Glc), D-galactose (Gal), D-mannose (Man), D-glucuronic acid (GlcA), *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetyl-D-galactosamine (GalNAc), L-fucose (Fuc), D-xylose (Xyl), D-ribose (Rib) and *N*-acetylneuraminic acid (Neu5Ac or NeuAc).
Mucin	Large glycoprotein with a high content of serine, threonine, and proline residues and numerous O-GalNAc-linked saccharides, often occurring in clusters on the polypeptide. Secreted by epithelial surfaces such as gastrointestinal, genitourinary and respiratory tracts.
N-(linked)glycan	Glycan covalently linked to an asparagine residue of a polypeptide chain in the consensus sequence: -Asn-X-Ser/Thr.
O-(linked)glycan	A glycan glycosidically linked to the hydroxyl group of the amino acids serine, threonine, tyrosine, or hydroxylysine. Glycoproteins rich in *O*-glycans are often called mucins.
Oligosaccharide	Linear or branched chain of monosaccharides attached to one another via glycosidic linkages. The number of monosaccharide units can vary.
PAMPs	See *Alarmins*.
Polysaccharide	Glycan composed of repeating monosaccharides, generally greater than ten monosaccharide units in length.
Saccharide	A generic term for any carbohydrate or assembly of carbohydrates, in free form or attached to another molecule, used interchangeably with carbohydrate and glycan.

Selectin	A C-type (Ca^{++}-dependent) lectin expressed by cells in the vasculature and bloodstream. The three known selectins are L-selectin/CD62L (expressed by most leukocytes), E-selectin/CD62E (expressed by cytokine-activated endothelial cells), and P-selectin/CD62P (expressed by activated endothelial cells and platelets).

Table 2

Glycosylation research in autoimmune diseases mimicked by cGVHD.

Author [reference]/year	Disease*	Key findings of the study
Dry mouth and eye disorders		
P. Youinou et al[80]/1992	Sjögren's syndrome	Elevated levels of asialylated IgG.
I. Castro et al[81]/2013	Sjögren's syndrome	Altered salivary mucins.
Gastrointestinal disorders		
J. M. H. Larsson et al[85]/2011	IBD	Reduced glycosylation of gastrointestinal mucins.
A. M. Dias et al[87]/2013	IBD	Aberrant N-glycosylation of T cell receptor.
I. Trbojevic Akmacic et al[23]/2015	IBD	Reduced immunosuppressive potential of IgG in IBD.
Hematological disorders		
T. Bakchoul et al[92]/2013	ITP	*N*-glycosylation of AAbs suggested to be prerequisite for platelet phagocytosis *in vitro* and *in vivo.*
Neurological and muscular disorders		
M. H. J. Selman et al[94]/2011	Myasthenia gravis	Lower levels of IgG2 galactosylation.
I. Perdivara et al[95]/2011	Myositis	Lower levels of IgG galactosylation.
Other		
C. Panzironi et al[83]/1997	SLE	'Incresead carbohydrate moiety' and higher concentration of galactose in α2-macroglobulin.
X.-X. Chen et al[84]/2015	SLE	Reduced IgG sialylation.
F. Vuckovic et al[24]/2015	SLE	Reduced immunosuppressive potential of IgG.

*IBD=inflammatory bowel disease; ITP= immune thrombocytopenia; SLE= systemic lupus erythematosus