Review

Aberrant glycosylation in autoimmune disease

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Received on May 31, 2019; accepted in revised form on August 6, 2019.

Clin Exp Rheumatol 2020; 38: 767-775.

Key words: glycosylation, autoimmune disease, glycoprotein, glycan, lectin, biomarker

Funding: this study was supported by the National Natural Science Foundation of China (81771780, 81660275), the CAMS Initiative for Innovative Medicine (2017-I2M-3-001), and the Guangxi Medical and Health Project (S2017022).

Competing interests: none declared.

ABSTRACT

Autoimmune diseases (AIDs) result in high levels of various autoantibodies in the serum as well as systemic inflammation and targeted organ damage. The incidence of AID has increased over recent decades. Glycosylation is a significant part of the post-translational modification of proteins and has been recognised as an important part of immune regulation in humans. Aberrant glycosylation manifests as pro- or anti-inflammatory effects. Numerous studies have confirmed that aberrant glycosylation plays a crucial role in the AID process. The development of emerging technologies such as the lectin microarray has facilitated research on the structure and function of glycans and glycosylation. Newly developed devices allow for high-throughput, high-speed, and highly specific research on aberrant glycosylation. Here, we review the role of glycosylation in the regulation of effector function in the context of autoimmunity and aberrant glycosylation in AIDs. This paper also discusses emerging technologies and clinical applications of glycosylation.

Introduction

The autoimmune diseases (AIDs) are a family of over 80 inflammatory disorders, in which the immune component of the host treats self-substance as a foreign invader, leading to a series of specific target organ damage, systemic disturbance, and even death (1). It has been shown that the incidence of AIDs has increased over the past two decades (2). Worldwide, 5–10% of humans may develop some type of autoimmune disorder throughout their lifetime (3-5). Several lines of clinical research have identified a common pathogenic mechanism of AIDs: once immune tolerance is broken, autoantibodies and autoreactive immune cells mediate an inflammatory response, resulting in pathological autoimmunity and eventually leading to organ and tissues damage (6-9). Nevertheless, the further development of AIDs requires the involvement of genetic susceptibility (10, 11) and the ability of environmental factors to act as triggers (12, 13). With the development of pathogenesis research and diagnostic experiments, mounting indicators provide evidence for elucidating the mystery of AIDs. The pathogenesis and early diagnosis of AIDs have attracted significant attention because early diagnosis and therapeutic management can help to prevent the onset of AIDs and associated irreversible damage (14, 15). Glycans are sugar molecules that adhere to glycoprotein. This group of molecules exhibits abundant structural diversity (16). It is known that almost all immune effector molecules are glycoproteins, including inflammatory mediators, immunoglobulins (Ig), and nearly 50% of serum proteins. Glycosylation is one of the most common and complex forms of post-translational protein modification. Glycosylation affects the biological functions of glycoproteins, such as the ability to maintain cell stability, the regulation of cellular activity, cell-cell adhesion, immunogenicity, and secretion (17, 18). Although the synthesis of glycans, as opposed to DNA and protein, does not require a template, structural modifications are mediated by glycosidases, glycosyltransferases, and sialidases. Numerous other factors regulate glycosylation, such as age, environmental factors, cell type and activation status, and inflammatory mediators (19, 20). All of these factors may be changed in the setting of inflammation and autoimmunity. In the context of inflammation and the autoimmune response, glycoprotein glycans may interact with immune effector molecules to foster the development of diseases characterised by specific glycosylation patterns. Each

disease thus has a specific glycosylation profile that can be viewed as an autoimmune signature. Mounting evidence has confirmed that the characteristic profile of glycosylation in AIDs can provide information related to disease pathogenesis and biomarkers that may be effective for use in the clinic.

In the following sections, we provide a review of recent developments in the field of experimental technology for glycomic analysis and the role of glycoprotein glycosylation in AIDs. Our purpose is to provide a comprehensive perspective from which researchers may develop glycosylation research to help in the treatment of AIDs. The findings presented below may encourage researchers to explore new approaches to therapeutic management based on the role of glycosylation in AIDs.

Glycosylation regulates immune effector functions

AIDs is characterised by the excessive production of autoantibodies, and aberrant glycosylation of antibodies has been shown to be involved in the pathogenesis of AIDs. The most abundant serum glycoprotein in humans is immunoglobulins (Ig), which are produced by B cells and plasma cells. Ig plays a crucial role in inflammation and autoimmunity. There are five types of Ig: IgG, IgM, IgA, IgE, and IgD. For IgG, the most abundant type of immunoglobulins, both the Fc (crystallisable fragment), in charge of modulating effector functions, and Fab (antigen-binding fragment), in charge of binding Ag, may undergo glycosylation.

Effects of glycosylation on IgG Fc functionality

IgG Fc fragments determine the effector function of IgG and the nature of the inflammatory response by binding to Fcγ receptors (FcγRs). FcγRs may be roughly categorised as either activating FcγRs (FcγRI, FcγRIIA, and FcγRIIIA) or inhibitory FcγRs (FcγRIIB) (21). Once accumulated IgG-Fc fragments have bound to activated FcγR, they induce cross-linking between two or more receptors, which triggers receptor signalling. Subsequent immune responses include antibody-dependent cellular cytotoxicity (ADCC), the regulation of antibody production, reactive oxygen species (ROS) production, secretion of inflammatory mediators, and phagocytosis (22, 23). IgG Fc glycosylation can alter the affinity of activated immune complexes (ICs). ICs are formed by IgG, interact with FcγR, and are expressed by recruited inflammatory effector cells, ultimately leading to disease onset, even tissue damage (24). The levels of IgG fucosylation has been shown to be play an important role in ADCC. Most circulating IgG is fucosylated (25). The addition of fucose glycan significantly reduces ADCC by reducing binding affinity between IgG and activated FcγRIII(23, 26). In addition, fucosylation seems to weaken antibody-dependent cell-mediated phagocytosis (27, 28). Furthermore, high levels of mannose structures affect IgG effector function. One study investigated the effects of human monoclonal antibodies containing oligomannose-type glycans on the presence of kifunensine, which improved ADCC function and affinity for FcγRIIIA but decreased C1q binding (29). Moreover, reduction of Fc sialylation also inhibited binding of the antibody to C1q (30). Thus, the process of Fc glycosylation implies a potential role in proinflammatory effector functions such as ADCC and complement-dependent cytotoxicity (CDC).

Effects of glycosylation on IgG Fab functionality

Although an IgG's Fc region dictates its effector function, antigen-specificity is determined by the Fab arm. The binding affinity of Fab arms may be affected by the presence of glycosylation in the variable domains. For instance, it has been confirmed that the removal of sialic acid glycans from Fab arms may decrease Ag-binding affinity (31). A 3–8-fold reduction in binding to CD33 was observed when N-linked glycans were present in the Fab arms of anti-CD33 antibodies (32). It is generally accepted that the proinflammatory efforts of IgG are achieved through activation of the complement system and stimulation of the ADCC effect. Fab arm glycosylation could influence antibodies aggregation and modulate the formation of immune complex. Researchers designed aggregation-prone regions on the Fab domain of a therapeutic monoclonal antibody, bevacizumab, to rationally design a biobetter drug candidate according to engineering single point mutations of aggregation-prone residues and glycosylation site near aggregation-prone residues to mask these residues with a carbohydrate moiety (33). In addition, Fab glycan could modulated half-life of serum glycoproteins and organ targeting through their position (34). The extent to which IgG Fc and Fab are glycosylated is associated with individual age and gender, respectively (35).

Effects of glycosylation on glycoprotein functionality

Approximately 50% of the proteins in serum are glycoproteins. Numerous proteins, other than immunoglobulins, are considered to play a role in the pathogenesis of AIDs. These proteins include α-1 acid glycoprotein (AGP), α-2 macroglobulin (AMG), haptoglobin (HAP), C-reactive protein (CRP), and transferrin (TFN), all of which are acute-phase proteins (APPs). The aberrant glycosylation status and modified glycan structure of APPs contribute significantly to the progression of AIDs. For example, increased levels of glucosamine, galactose, mannose, fucosylated AGP, and sialyl Lewis-x (sLe^x), were observed in rheumatoid arthritis (RA) (36-38). Abnormal glycosylation of APPs contributes to the pathogenesis of RA by increasing fucosylation, mannosylation HAP (38), and branching TFN (39). AIDs are also associated with increased binding to some lectins contained in APPs. In RA, with secondary Sjögren syndrome (SS), and systemic lupus erythematosus (SLE), alterations in the pattern of glycosylation result in increased reactivity to concanavalin A (Con A) (40, 41). In the contexts of inflammation and the autoimmune response, aberrant glycosylation in APPs results in high levels of fucosylation, sialylation, and mannosylation, as well as increased expression of sLe^x in branched glycans (42).

In addition to serum proteins, others that are expressed or secreted by particular organs or tissues are thought

to be involved in the pathogenesis of AIDs. A study of parotid saliva from patients with SS showed that measurements of *Dolichos biflorus* agglutinin (DBA)-glycan-specific binding activity may provide a more specific indicator of salivary gland disease than measurements of lactoferrin concentration alone (43). Nayab *et al*. proposed that levels of MUC7 (a secreted mucin) glycosylation may be an index for saliva quality in patients with SS (44).

Recent progress in tools for the analysis of glycosylation

The analytical procedures required for measuring glycan levels, identifying sites of glycosylation, and characterising glycan structure are challenging and complicated. Additional research on the effects mediated by glycoproteins and glycans is urgently needed. The results obtained will inform disease diagnosis and may have therapeutic utility. The general idea of glycosylation analysis is based on the following premise. Considering that the state of glycoprotein glycosylation changes in parallel with the disease process, target glycoproteins may be extracted and enriched to investigate the degree to which a specific disease has progressed (45). After extraction and enrichment, laboratory methodology will be used to dissociate the glycan from the glycoprotein. The glycans obtained in this fashion will then be analysed using mass spectrometry (MS) (46), capillary electrophoresis (CE) (47), and lectin-based enzyme-linked immunosorbent assay (ELISA) (48). Use of these research strategies has revealed mounting evidence of aberrant glycosylation in association with AIDs (as described in 3).

MS/MS-based analysis of glycosylation

Most studies on AID glycosylation performed to date have been based on the use of MS. One common approach to the measurement of glycosylation includes the preliminary capture of glycans and glycopeptides, followed by the analysis of glycans using MS and profiling to characterise the glycan and glycopeptide structures collected. Integrated glycans may be captured with hydrophilic interaction chromatography (HILIC) enrichment (42, 49), lectin affinity enrichment (50), or mixed anion exchange (MAX) extraction (51). The MAX method was considered to be superior for capturing for native N-linked glycans and N-terminal–labeled intact glycans. Several types of MS have been used to resolve integrated glycans. The residues may be distinguished using vibrational energy-based CID or HCD, electronic excitation-based ETD, or ECD. These methodological approaches provide highly complex spectra. Several software programmes have been developed to analyse the MS spectra associated with various patterns of glycosylation. These software programs include Glycopep Grader (52), GPQuest (53, 54), Glycopeptide Search (GPS) (55), and pGlyco (56, 57). Although MS/MS-based methods have advantages for analysing glycan structure, but the glycan enrichment step requires large quantities of purified glycoprotein (on the range of micrograms or milligrams). MS analysis is not conducive to broad use in clinical applications.

An emerging lectin microarray technique for glycosylation analysis

Lectin microarray is an emerging and high-throughput method that has long been used to measure levels of glycosylation in purified glycoproteins, serum, live cells, sperm, and various other tissue types (58-62). Lectin microarrays, which consist of nearly 100 lectins, may be used to reliably identify specific binding glycans. Lectin microarrays allow for fast and high-throughput glycan profiling. Compared with the MS method, use of a lectin microarray does not require many specimens. Use of only a small amount of material (*e.g*. 1 nanogram) allows for the reliable characterisation of lectin binding characteristics (63, 64). Using the lectin microarray, Shinzaki (65) *et al*. confirmed that both *Agaricus bisporus* agglutinin (ABA) and *Griffonia simplicifolia* lectin-II (GSL-II) preferentially recognise agalactosylation IgG. The binding affinity of each of these lectins for IgG is significantly elevated in Crohn disease (CD). Additional studies performed using the lectin-antibody enzyme immunoassay (EIA) confirmed that agalactosylation IgG may be used as a serum marker for inflammatory bowel disease (IBD). Li *et al*. (62) showed that a two-phase platform combining lectin microarray and lectin-based immunosorbent assays may be used to diagnose aggressive prostate cancer or to measure glycosylation in tissue samples from patients with prostate cancer as well to diagnose aggressive prostate cancer. These results point to a role for the lectin microarray in the analysis of specific binding between lectins and glycans and in the measurement of glycosylation. When used in combination with other techniques, the lectin microarray may be used to elucidate the pathogenesis and diagnosis of AIDs.

The role of glycosylation in AID

Aberrant glycosylation is an essential characteristic of immune disorders such as AIDs. The ability to delineate the glycosylation patterns of glycoproteins associated with AIDs may allow researchers to identify changes that are specific to disease onset and thereby improve efforts at disease management. Aberrant glycosylated MPO may react with antibodies in most patients diagnosed with anti-GBM disease without MPO-ANCA. These findings suggest that the abnormal glycosylation of MPO molecules may expose neoepitopes to the immune system (66). One previous study described decreased oxidation and a microbicidal effect of deglycosylated MPO in ANCA-associated vasculitis, demonstrating the effects of glycoprotein glycosylation on inflammation and the autoimmune response (67). Studies of protein glycosylation have been identified as a potential tool for elucidating the pathogenesis of AIDs as well as the optimal approach to disease management.

Disease pathogenesis

• *Aberrant IgG glycosylation in AIDs*

IgG galactosylation is decreased in RA (68-71). In patients with RA, levels of the agalactosyl glycoforms of IgG (IgG-G0) are increased and positively associated with levels of disease activity (70, 71). Levels of this glycoform

are increased in AIDs, SLE, SS, Wegener's granuloma (WG), and microscopic polyarteritis (MPA) (72-74). Studies in patients with RA have revealed a relationship between increased levels of IgG-G0 and decreased levels of galactosyltransferase (GTase) activity in B-cells (75). The resection of terminal galactoses of human IgG increases the uptake of soluble IgG, which is mediated by the mannose receptor on dendritic cells (DCs) and macrophages (76). This form of uptake may represent a pathway by which autoantibodies and ICs can be incorporated into macrophages and DCs, leading to the production of antigen epitopes by T-cells (73). IgG-G0 is structurally susceptible to insufficient numbers of sialic acid residues, which has a pro-immune effect in AIDs (77). Similarly, the IgG-G0 glycopattern revealed that core-fucosylation levels of IgG were unaffected; however, increased levels of core-fucosylation may enhance the effect of ADCC (78, 79).

• *Glycosylation of disease biomarkers in AIDs*

Recent studies have clarified the specific autoantigen reactivity of autoantibodies produced by patients with AIDs, with implications for the diagnosis of disease. Magorivska compared the glycans in native IgG between seropositive RA and seronegative RA using capillary electrophoresis with laser-induced fluorescent detection (CE-LIF). The results obtained by the authors showed that the proportion of glycans differed between seropositive and seronegative RA because of the specific autoantibodies present in patients with seropositive RA (80). Several studies also revealed that glycosylation of anti-citrullinated protein antibodies (ACPAs), an RA-specific biomarker, has a critical effect on diseases onset (81, 82). ACPA-IgGs were isolated from RA patients by affinity purification using fast protein liquid chromatography (FPLC). Structural analysis by MS showed that a high proportion of ACPA IgG from RA patients could be distinguished by the presence of additional N-linked glycans in the variable domains. The presence of additional N-linked glycans was caused by somatic hypermutation at the N-linked glycosylation site, which facilitated binding between aberrant glycans and citrullinated antigens (81). This alternative advantage to ACPA-producing B-cells also suggests a pro-immune role for hyperglycosylated ACPA IgG molecules in RA.

Rombouts *et al*. also investigated ACPA glycosylation with affinity purification and cleavage of ACPA IgG from RA serum. Analysis of serum ACPA-IgG1 levels before the onset of RA revealed decreased levels of galactosylation and increased levels of core fucosylation (82). The same changes were observed for ACPA in synovial fluid, indicating a strong IgG-G0 pattern (83). Although the biological processes involved in these results remain to be elucidated, they imply a relationship with binding to FcγR. The authors observed decreased binding affinity of ACPA for FcγRIIIa and FcγRIIb, compared with other activating receptors (*e.g*. high-affinity FcγRI) (26, 84, 85). This finding suggests that reduced affinity for FcγRIIIa and FcγRIIb may increase the effect of ACPA binding to FcγRI, further enhancing pro-immune effects. In combination with a previously reported finding that IgG Fc glycosylation is regulated by various extracellular factors in vitro, this report of aberrant glycosylation in ACPA-IgG1 highlights the crucial role of the microenvironment for IgG Fc glycosylation (86).

Anti-histone antibodies have been reported to be involved in the recruitment of polymorphonuclear cells (PMN) to destroy apoptotic cells. After the activation of phagocytes, autoantibody-loaded secondary necrotic cells (SNEC) trigger an immune response (87, 88). Anti-histone IgG in serum from SLE patients has high levels of asialylation and may thus be distinguished from total IgG from the same patients (89). The binding affinity of sialylated antibodies for FcγRIIB is decreased (77). A lack of sialic acid residues in anti-histone antibodies may alter the pattern of binding to FcγR, resulting in a pro-inflammatory effect. The presence of asialylated anti-histone antibodies in SLE patients may be related to tissue damage because of the targeting of SNEC towards PMN.

Another study was conducted to investigate the role of anti Jo-1 autoantibody glycosylation in inflammatory myopathies (IIM) and anti-synthetase syndrome (ASS). Significant decreases in bisected, afucosylated, and galactosylated glycopatterns were observed in anti Jo-1 IgG Fc samples from patients with ASS or IIM, compared with serum total IgG from the same patients (90). Although the particular mechanism underlying aberrant glycosylation of disease-specific autoantibodies with AIDs in disease pathogenesis remains to be elucidated, it is certain that the glycan residues attached to the autoantibodies affect the affinity of autoantibodies, reaction with FcγR, participation in complement activation, and the secretion of cytokines (91). An overview of aberrant glycosylation in the pathogenesis of AIDs is given in Table I.

Disease activity

Researchers have proposed that abnormal glycosylation of autoantigen-specific autoantibodies may play a role in the pathogenesis of AIDs. Elevated G0 IgG levels are a characteristic feature of RA and associated with disease activity (92-95). In addition, levels of serum total sialic acid (TSA) and free sialic acid (FSA) were increased in RA patients, compared with systemic sclerosis (SSc) patients, SLE patients, and heathy individuals; levels of TSA and FAS were positively correlated with the RA disease activity index (DAS28) (96). The pathogenic role of aberrant glycosylation in RA may be due to increased affinity for FcγR when IgG molecule terminals lack galactose residues and sialic acid residues (97, 98). Instead, the adherence of terminal sialic acid residues to IgG could reduce the affinity of autoantibodies to FcγRs and further enhance the anti-inflammatory effect of IgG-G0 (77, 99).

Serum IgG4 concentration level is abnormally elevated in patients with IgG4-related disease (IgG4-RD). As one of the subtypes of IgG molecules, IgG4 was confirmed to has aberrant glycosylation in IgG4-RD. Naok *et al*. found that IgG4 G0 N-glycans and IgG4 fucosylated N-glycans in IgG4-RD when compared with healthy con-

Table I. Aberrant glycosylation changes in the pathogenesis of AIDs.

Disease	Aberrant glycosylation	Reference
RA	Decreased serum IgG galactosylation, increase in IgG G0	(68-71)
	Decreased ACPA-IgG galactosylation, increase in IgG G0	(81-83)
	increase in serum matrix metalloproteinase-3 α-2,6-sialylation	(127)
	Increased in acute-phase proteins galactosylation and fucosylation	(36-38)
SLE	Increased $CD4^+$ T cells core fucosylation	(128)
	Decreased serum IgG sialylation	(74, 89, 96)
	Decreased serum IgG galactosylation and core fucosylation, increased bisecting N-acetylglucosamine structure	(74)
	Increase in *Aleuria aurantia* lectin- and *Lens culinaris* agglutinin reactive glycans in serum IgG	(48)
	Increased alpha 2-macroglobulin galactosylation	(41)
AAV	Decreased serum IgG galactosylation and increase in IgG G0	(73, 110, 129, 130)
	Decreased anti-PR3 ANCA galactosylation, sialylation and bisection	(107, 110)
pSS	Decrease in the extended core 2 disialylated structure and fucosylated core 2 disialylated structure in mucins MUC7	(44)
	Decreased mucin sialylation in saliva	(131)
	Increased numerous salivary glycoproteins N-glycosylation	(132)
	Increased both IgA1 Fc- and Fab-sialylation	(133)
IIM	Increase in pro-inflammation associated IgG Fc-glycans, decrease in bisected, afucosylated and galactosylated anti-Jo1 antibody	(90)
	Increase in core-fucosylated agalactosyl glycans	(134)
IgG4-RD	Decreased IgG-Fc galactosylation, increased IgG Fab sialylation and IgG4 fucosylation	(101)
	Increase in IgG4 G0 N-glycan and IgG4 fucosylated N-glycan, decreased levels of IgG4 F0 glycan in IgG4-RD with hypocomplementaemia.	(100)

trols, with IgG4 non-fucosylated N-glycans decreased in those with hypocomplementaemia (100). Subsequently, Emma *et al*. confirmed that IgG4 fucosylation negatively correlated with C3 and C4 levels in patients with IgG4-RD (101). These results indicated that IgG4 fucosylation correlated with disease activity according to alteration between complement-mediated and IgG FcγR-mediated effector function (102).

Additional studies were conducted to investigate glycosylation in the context of pregnancy-related RA. Results obtained by MS revealed significant differences between pregnant patients with RA and controls in levels of galactosylation and sialylation in the ACPA-IgG Fc domain. Furthermore, the increase in galactosylation observed in ACPA-IgG–positive patients was associated with lower levels of DAS28-CRP (46). Interestingly, the association between galactosylation of the ACPA-IgG Fc domain and disease activity in pregnant RA patients was limited to the Fc domain of ACPA-IgG. This trend was not observed in analysis of serum total IgG, IgA, or IgG Fab segments (46, 103, 104). One possible mechanism for pregnancy-specific changes in ACPA-IgG glycosylation is that the increase in levels of estrogens decreases aberrant glycosylation, especially aberrant galactosylation (105). Other important factors include the secretion of cytokines [*e.g*. interleukin (IL)-6] by immune cells which further promotes up-regulation of estrogens receptors and exerts a pro-inflammatory effect (106).

Another study showed that the sialylation level of anti-PR3 autoantibodies was significantly decreased in patients with active GPA and negatively correlated with the Birmingham vasculitis activity score (BVAS)(107). In one study that involved use of MS for structural analysis, purified IgG from active GPA was compared with purified IgG from non-active GPA and from control subjects. The results showed decreased levels of 2,6-linked sialylated N-glycans and increased levels of agalactosylated glycans in purified IgG from active GPA. This implied that the sialylation level of anti-PR3 antibodies may be used as a marker of disease activity in GPA.

Binding to *Aleuria aurantia* lectin (AAL) and *Lens culinaris* agglutinin (LCA) was increased in the IgG complexes of patients with SLE compared with healthy controls, and high AAL binding activity was related to the level of disease activity (48). IgG-capture lectin ELISA analysis revealed that the binding of immobilised IgG complexes to lectin involves the fucosylated residues attached to the IgG. High levels of AAL-fucosylated residue activity is positively associated with disease activity and negatively associated with levels of circulating C3 in patients with SLE (48). Ahn reported that serum levels of Mac-2-binding protein, which binds specifically to *Wisteria floribunda* agglutinin (WFA) and may be captured by with a WFA-positive-M2BP ELISA, reflect levels of disease activity in patients with SLE (108). These conclusions suggest that the glycosylation status of glycoproteins may be used as a marker of disease activity in AIDs.

Disease relapse

ANCA are pathogenic autoantibodies found in patients with AAV, levels in serum appear to be associated with disease activity (109). However, this association is not clear enough to predict clinical outcomes and risk for disease relapse (110). GPA patients with low levels of total IgG1 galactosylation and sialylation were more likely to relapse after an increase in ANCA levels.

Compared with non-relapsing patients, relapsing patients showed high levels of fucosylation and changes in total IgG1, including decreases in galactosylation, sialylation, and bisected structures. Levels of PR3-ANCA IgG1 did not differ between relapsing and non-relapsing GPA patients; both groups had decreases in the galactosylation, sialylation, and fucosylation of PR3-ANCA IgG1 (110). Further research will be necessary to explore the relationship between the glycosylation state of disease-specific autoantibodies and disease relapse in patients with AIDs.

Disease therapy

An effective anti-inflammatory therapeutic is required to slow the progress of inflammation. Biologic therapies, especially anti-tumor necrosis factor (TNF)-α therapy, have been used widely in the treatment of AID. When combined with chemically synthesised disease-modifying anti-rheumatic drugs (DMARDs) in the treatment of AIDs (mainly SLE and RA), TNF antagonists may suppress binding to the TNF-α receptor, which blocks the cell signalling mediated by these pathogenic cytokines and improves the secretion of anti-inflammatory factors (111, 112). Anti-TNF-α treatment may also reduce IgG-G0/G1 levels to normal range (113-117). Effective biologic treatment for RA is also associated with decreased levels of sialylated triantennary glycans and increased levels of core-fucosylated biantennary galactosylated glycans (114).

The pro- and anti-inflammatory effector functions of IgG are mediated by different subclasses of IgG molecules and various patterns of Fc glycosylation. IVIG (pooled human serum IgG from healthy donors) is widely used in the treatment of AIDs and inflammatory diseases (77, 118, 119). Sialylated IgG has an anti-inflammatory effect on the immune response in humans. Because of the sialylation of IVIG, high doses (2 g/kg) may be used to treat patients with AIDs (77, 118, 119). Research in a mouse model showed that sialylated IVIG binds to specific ICAM-3 grabbing non-integrin-related 1 (SIGN-R1), a C-type lectin receptor on marginal-zone macrophages, to attenuate the onset of arthritis (120). This binding activity leads to an anti-inflammatory microenvironment and up-regulates the expression of inhibitory FcγR (especially FcγRIIB) on functional macrophages. Furthermore, sialylated IVIG inhibits dendritic cell (DC) maturation through an FcγRIIB signalling pathway (121-124). ICs containing sialylated antigen-specific IgG antibodies limit the production of IL-6 induced by lipopolysaccharide (LPS) in DCs *in vitro* (121). Finally, endogenous sialylated IgG molecules exhibit decreased disease activity in mouse models of nephritis and arthritis. The underlying mechanism is thought to be similar to that responsible for the effects of IVIG (125).

Researchers have also studied the effect of low levels of sialylated autoantigen-reactive IgG antibodies on inflammation in mouse models (126). One study showed that treatment with sialylated autoantigen-reactive IgG antibodies did not induce inflammation or lupus nephritis in a mouse model of lupus. However, treatment with sialylated autoantigen-reactive IgG antibodies decreased the magnitude of pathogenic Th1, Th17, and B-cell responses. Nonetheless, treatment with these antibodies may alleviate disease symptoms and reduce the number of pathogenic Th17 cells and autoantigen-specific IgG antibodies (126). Indeed, the sialylation of pathogenic antibodies could effectively attenuate inflammation *in vivo*. Researchers have designed solubilised glycosyltransferases that attach sialic acid to autoantibodies (125). The results obtained in subsequent experiments suggest that sialylated autoantigen-reactive IgG antibodies may attenuate pathogenic T- and B-cell-induced immune responses in patients with AIDs.

Concluding remarks and future prospects

As an effective mediator of AID, measurements of protein glycosylation may be used to elucidate pathogenesis, diagnose disease, manage disease activity, and effectively treat patients with AIDs. The occurrence and development of AIDs is accompanied by the aberrant glycosylation of glycoproteins in the immune system. Emerging experimental methods, which may benefit from the rapid development of detection technology, are currently used to analyse the glycosylation status of glycoproteins and the structure of glycans. Increasing numbers of clinical studies have reported the effector function of glycosylation in AIDs. High-throughput experimental techniques such as lectin microarray can quickly screen for glycans that bind specifically to lectin in specimens from human individuals. This implies that lectin microarray may play a potential role in individualised therapy for AIDs.

Therapeutic strategies for glycosylation seem to be a hot issue. Booming knowledge of how Fc glycans conform to IgG structure and effector functions has opened up a new field for the therapeutic development of defined antibody glycopatterns in the treatment of AIDs. The crucial role of Ig glycosylation in the effector function of autoantigen-reactive IgG antibodies may now be considered a remarkable target for the development of new AIDs therapeutics.

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