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Review

IgG antibodies are secreted from B cells and bind to a variety of pathogens to control infections as well as contribute to inflammatory diseases. Many of the functions of IgGs are mediated through Fcγ receptors (FcγRs), which transduce interactions with immune complexes, leading to a variety of cellular outcomes depending on the FcγRs and cell types engaged. Which FcγRs and cell types will be engaged during an immune response depends on the structure of Fc domains within immune complexes that are formed when IgGs bind to cognate antigen(s). Recent studies have revealed an unexpected degree of structural variability in IgG Fc domains among people, driven primarily by differences in IgG subclasses and N-linked glycosylation of the CH2 domain. This translates, in turn, to functional immune diversification through type I and type II FcγR–mediated cellular functions. For example, Fc domain sialylation triggers conformational changes of IgG1 that enable interactions with type II FcγRs; these receptors mediate cellular functions including antiinflammatory activity or definition of thresholds for B cell selection based on B cell receptor affinity. Similarly, presence or absence of a core fucose alters type I FcγR binding of IgG1 by modulating the Fc's affinity for FcγRIIIa, thereby altering its proinflammatory activity. How heterogeneity in IgG Fc domains contributes to human immune diversity is now being elucidated, including impacts on vaccine […]

Functional diversification of IgGs through Fc glycosylation

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Introduction

IgG antibodies act as a bridge between the host and foreign antigens, coupling antigen detection with the recruitment of innate and adaptive immune processes. This capacity arises from the presence of two functional domains: the antigen-binding Fab domain and the Fc domain, which interacts with Fcγ receptors (FcγRs) to mediate an array of cellular effector functions (1, 2). Diversification of IgG-mediated effector functions is achieved by structural variation in Fc domains; Fc domain structure determines the FcγRs, and in turn the effector cells, that can be engaged. One critical determinant of Fc structure that can impact both adaptive and innate FcγR signaling pathways is glycosylation of the Fc. In this Review, we will discuss how Fc sialylation and fucosylation impact the functionality of IgG1 antibodies as well as existing and potential clinical applications for IgGs with specific glycan modifications.

The activity of IgG antibodies depends on both their IgG subclass and Fc glycosylations (3–6). IgGs are found in four subclasses (IgG1–4) in humans, with IgG1 and IgG3 having the highest affinity for activating type I FcγRs. Aside from subclass, Fc structure is further defined by the precise composition of a complex, biantennary N-linked glycan present at Asp297 of each CH2 domain (Figure 1, upper left). A core glycan is always present, composed of seven saccharide units: four *N*-acetylglucosamine and three mannose residues. Removal of the core glycan diminishes affinity of the Fc for FcγRs, translating to loss of FcγR-mediated effector functions in vivo (7–13). Despite the requirement of Fc glycosylation for Fc-FcγR interactions, the molecular interactions between the Fc and FcγRs are mediated primarily by amino acid residues rather than saccharide residues (14). One exception to this is afucosylated Fc glycoforms, which stabilize interactions with FcγRIIIa and FcγRIIIb via a sugar-based interaction (15). Generally, the role of the Fc glycan is in regulating structure of the Fc by determining the degree of its conformational stability and thus interactions between the Fc and type I or type II FcγRs (9, 13, 16–18). The ability of the Fc to adopt distinct structures arises from its organization into two chains, each composed of two domains, termed CH2 and CH3. The two chains of the IgG heavy chain that make up the Fc are bound by disulfide bonds in the hinge-proximal CH2 region without extensive protein-protein interactions between the CH3 domains, resulting in a horseshoe-like configuration for the dimeric structure. Absence of additional bonds between the CH2 or CH3 domains of the Fc results in its capacity for conformational flexibility.

The core Fc glycan can be modified by specific saccharide units, including core fucosylation, bisecting GlcNAcylation, and galactosylation at one or both arms; the presence of galactose enables addition of terminal sialic acid(s). The terminal sialic acid modification, in the presence of core fucose, destabilizes the IgG1 Fc, enabling sampling of a "closed" Fc conformation and associ-

Conflict of interest: TTW is a named inventor on a patent (10,300,127) and patent applications relating to IgG Fc effector functions and glycan modifications. JVR is a consultant or member of the scientific advisory board of Harpoon, Kleo, Momenta, Palleon, Portola, Vir, and Xencor and receives consulting fees and stock options. He also has stock holdings in Macrogenics. He is a named inventor on patent numbers: 10,300,127; 10,167,332; 9,845,358; 9,657,101; 9,587,025; 9,481,724; 9,134,310; 8,815,237; 8,618,251; 8,470,318; 7,846,744; and 7,416,726 and on patent applications relating to Fc modifications.

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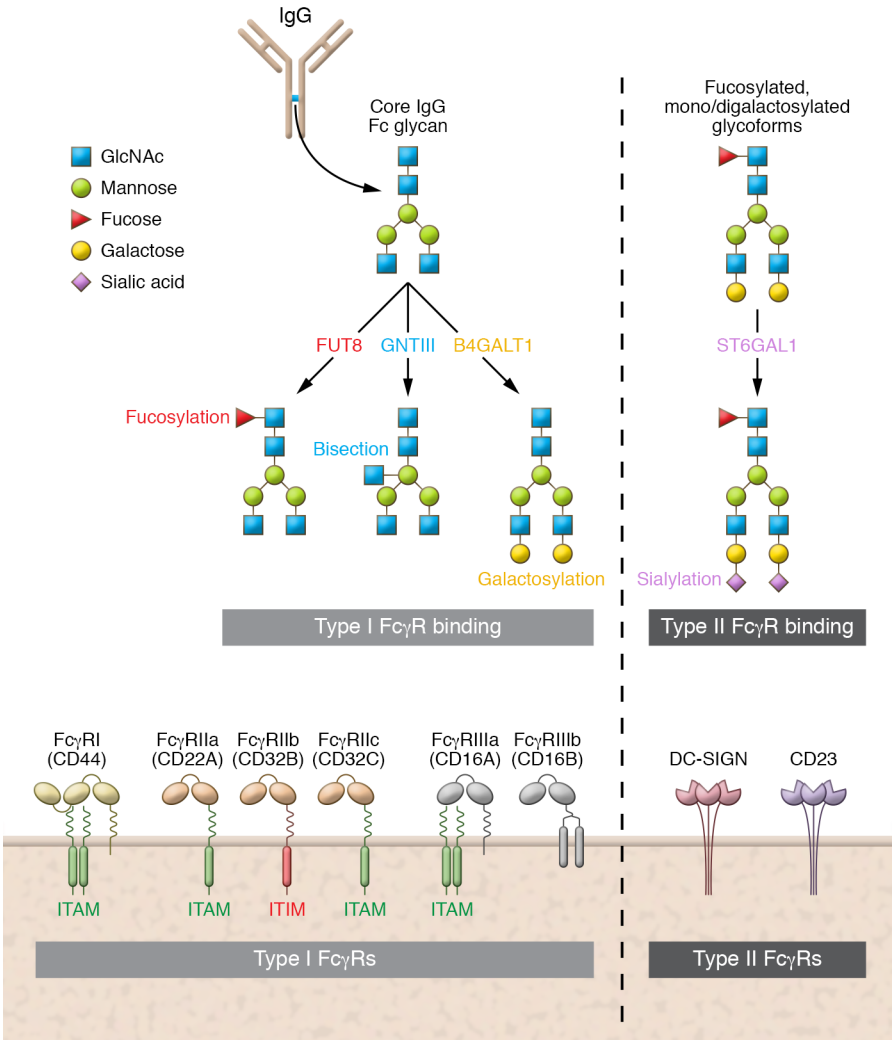

Figure 1. The core IgG Fc glycan modifications and type I and type II FcγRs. Top: The core Fc glycan is attached within the CH2 domain of each IgG heavy chain and can be modified by various glycosyltransferases for addition of fucose (FUT8), galactose (B4GALT1), *N*-acetylglucosamine (GNTIII), and sialic acid (ST6GAL1) residues. Although sialylation without fucosylation does not impact the enhanced binding of the afucosylated glycoforms to the type I FcγR FcγRIIIa, sialylation of fucosylated glycoforms destabilizes the Fc domain, enabling structural rearrangement that favors type II FcγR binding. Bottom: Type I FcγRs are members of the immunoglobulin super family and transduce activating or inhibitory signaling on the basis of the presence of an intracellular immunoreceptor tyrosine-based activation motif (ITAM) or immunoreceptor tyrosine-based inhibitory motif (ITIM) motif. Type II FcγRs are the C-type lectins DC-SIGN and CD23, which mediate antiinflammatory activity and B cell modulatory activities, respectively. Type II FcγRs are distinguished by their ability to engage sialylated, fucosylated immune complexes.

ation with type II FcγRs (Figure 1, upper right and refs. 9, 17, 18). Absence of core fucose enhances affinity of the Fc for the type I FcγRIIIa through interactions between the carbohydrate moieties on FcγRIIIa and the IgG1 Fc (15); this occurs regardless of sialylation status (17). While each of these saccharide modifications likely plays a role in regulating the Fc-FcγR interactions of various IgG subclasses their activities have been best studied in the context of the IgG1 subclass. As little is known about the impact of sialylation or fucosylation on IgG2–IgG4 at this time, discussions in this Review refer specifically to sialylation and fucosylation of IgG1 antibodies.

Fcγ receptors

Type I and type II FcγRs are distinguished by their ability to interact with Fcs based on specific glycan modifications. Type I FcγRs (FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, FcγRIIIb) are members of the immunoglobulin superfamily and show reduced binding to sialylated Fcs relative to those without sialic acid modification (19), while the absence of fucose uniquely enhances FcγRIIIa and FcγRIIIb binding (FcγRIIIb is a low-affinity type I FcγR without a signaling domain) (20). Recruitment of effector cells through Fc–type I FcγR interactions is required for a major subset of antibody-mediated functions, including those involved in protection against many infectious diseases and in tumor immunotherapy (21–28). Type II FcγRs are more recently described and include human dendritic cell–specific ICAM3-grabbing non-integrin (DC-SIGN/CD209) and CD23 (FcεRII), both C-type lectin receptors that can be engaged by sialylated Fc domains within immune complexes (Figure 1, bottom and refs. 9, 18, 29, 30). Type II FcγRs mediate distinct modulatory activities including antiinflammatory signaling (DC-SIGN) (30–32) and modification of selection of B cells in the germinal center (CD23) (29), and likely mediate additional activities yet to be defined. In addition to DC-SIGN and CD23, sialylated Fcs may interact with other C-type lectin receptors such as CD22, CLEC4M, CLEC4G, and CLEC4A (DCIR), but the biological significance of those interactions is not yet known (33–35).

DC-SIGN is expressed on dendritic cells and on subsets of monocytes/macrophages and B cells (36–38). While DC-SIGN can act as an FcγR, it can also engage other ligands, including a variety of pathogen-associated glycoproteins (39–43). Binding of DC-SIGN to these ligands occurs predominantly through carbohydrate-mediated interactions. This contrasts with the mechanism of sialylated immune complex–DC-SIGN interactions, which occur through protein-based interactions in a region of the Fc that becomes exposed upon sialylation (9, 18, 44).

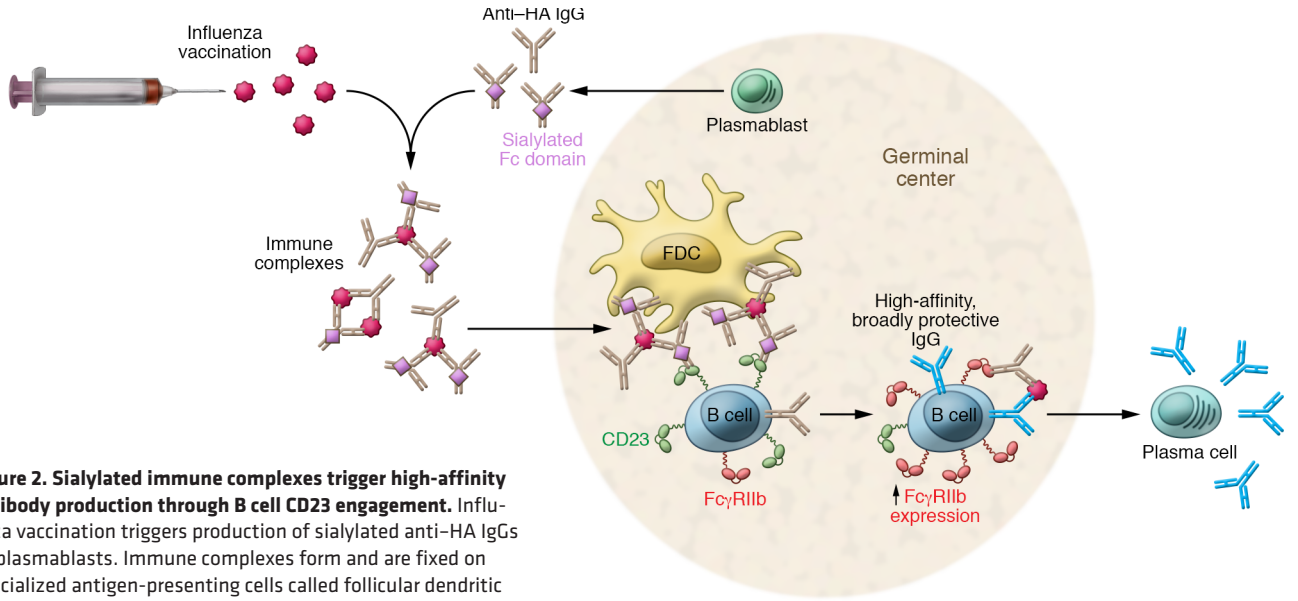

Figure 2. Sialylated immune complexes trigger high-affinity antibody production through B cell CD23 engagement. Influenza vaccination triggers production of sialylated anti–HA IgGs by plasmablasts. Immune complexes form and are fixed on specialized antigen-presenting cells called follicular dendritic cells (FDCs) within the germinal center. Coengagement of CD23 with the B cell receptor by sialylated HA immune complexes induces increased expression of the inhibitory FcγRIIb, resulting in selection of higher-affinity B cells.

CD23 exists as two splice variants, CD23a and CD23b, which vary by cellular expression and the ability to be induced by IL-4 (45). CD23a is constitutively expressed on B cells, while CD23b expression is induced by IL-4 and is limited to myeloid cells and some T cell subsets (45). CD23 was originally described as the low-affinity receptor for IgE and has been proposed to function in regulation of IgE synthesis (46). The ability of CD23 to function as both an IgE and IgG Fc receptor was first suggested by modeling studies showing a high degree of structural homology between the proposed IgE-CD23 and sialylated IgG Fc–CD23 complexes (9, 47, 48). Subsequent in vitro and in vivo studies confirmed that CD23 can act as a receptor for IgG (9, 29). As with DC-SIGN, sialylated immune complex–CD23 interactions are thought to occur through a mechanism of protein-based interactions that depend on flexibility of the IgG CH2 domain and conformational changes that occur upon Fc sialylation (9, 18).

With the exception of FcγRI, other type I and type II FcγRs have low affinity for Fcs such that Fc-FcγR interactions take place primarily in the context of immune complex formation, when avidity-based interactions can occur. This requisite immune complex formation confers specificity to the effector cell response, but which FcγRs and effector cells can be engaged by a given immune complex is determined by the structure of Fc domains in the complex. For example, immune complexes that are rich in IgG1 and IgG3 subclasses will recruit activating cellular functions over those rich In IgG2; immune complexes rich in fucose and sialic acid will mediate modulatory/anti-inflammatory activities relative to those with reduced fucose and sialic acids. Thus, the specific distribution of IgG subclasses and posttranslational modifications of Fcs that is produced by an individual will determine the quality of antibodies produced by vaccination or after infection and is likely a central determinant of pathogenicity of autoantibodies.

Inflammation

Balanced signaling through activating and inhibitory FcγRs is required for inflammatory responses that contribute to health rather than to disease. A central mechanism for regulation of inflammatory responses is structural diversification of the IgG1 Fc via glycosylation. Fucosylation and sialylation are the two primary Fc modifications that impact recruitment of inflammatory effector cell responses. Reduced core fucosylation or reduced sialylation of the Fc promotes increased activating FcγR signaling and, in turn, enhances inflammatory effector cell activity. Core fucosylation regulates interactions with the activating type I FcγR, FcγRIIIa, with absence of a core fucose moiety resulting in stabilization of the Fc with FcγRIIIa and enhanced signaling. In contrast, Fc sialylation is destabilizing, leading to sampling of the "closed" Fc conformation, which diminishes type I FcγR interactions and enables interactions between the Fc and type II FcγRs (Figure 1 and refs. 9, 18, 19, 29–31).

Destabilization of the Fc upon sialylation leading to the "closed" Fc conformation was originally demonstrated through biochemical methods and by crystallization studies (9, 18, 19). While the dependence of effector functions mediated by sialylated Fcs on type II FcγRs is readily demonstrated in vivo, interactions between sialylated Fcs and type II FcγRs in vitro can be more sensitive to specific assay conditions than are Fc–type I FcγR interactions. This may be due to alternation between the "open" and "closed" Fc conformations upon sialylation, resulting in reduced stabilization and affinity for type II FcγRs. The dependence of activities conferred through sialylated Fcs on type II FcγRs has been confirmed in numerous studies using mice that do not express DC-SIGN/CD23. In the case of DC-SIGN, transgenic expression of the human DC-SIGN receptor in the SIGN-R1 (murine DC-SIGN ortholog) knockout mouse model rescues the biological activity associated with sialylated Fc administration (29, 30, 44, 49).

Shifts in signaling through different type I and type II FcγRs can have considerable clinical consequences. For example, increased activating type I FcγR signaling due to reduced fucosylation of IgG is associated with progression to severe disease in secondary dengue infections (50) and has been implicated in neonatal alloantibody-mediated disease (51–53). Promoting type I FcγR signaling can also have pronounced therapeutic effects as observed in the context of antibodies that mediate broad immunity against influenza viruses (25, 54, 55). Another important example is the improved protective activity that can be achieved by engineering of tumor immunotherapies toward activating type I FcγRs. For example, outcomes of anti-CD20 immunotherapy in chronic lymphocytic leukemia patients are improved by glycoengineering of the antibody to engage the activating FcγRIIIa with higher affinity (56). Further, recent clinical studies found that progression-free survival after anti-HER2 immunotherapy for metastatic breast cancer is significantly improved by engineering of the anti-HER2 mAb for enhanced activating type I FcγR binding affinity and reduced affinity for the inhibitory FcγRIIb (57). This improved survival after administration of the Fc-optimized anti-HER2 mAb may involve a vaccinal response whereby administration of an anti-tumor mAb mediates processing and presentation of the mAb's target protein, promoting maturation of T cells against the target, which can provide long-term protection against tumor growth (58).

Shifts in FcγR binding that increase type II FcγR–mediated activities occur in the presence of IgGs with sialylated Fcs. This can result in antiinflammatory activity, as observed with administration of high-dose intravenous immunoglobulin (IVIg). IVIg is purified IgG, pooled from thousands of donors, and has long been observed to mediate therapeutic activity in the setting of acute inflammatory diseases such as immune thrombocytopenia and chronic inflammatory demyelinating polyneuropathy. The antiinflammatory activity in IVIg arises from the fraction of IgGs within the pool that are modified by sialylated Fc glycoforms (6). Mechanisms involved in antiinflammatory activity of sialylated IgGs have been dissected in studies using mice expressing humanized DC-SIGN, the type II FcγR required for antiinflammatory activity of IVIg. These studies showed that DC-SIGN can transduce antiinflammatory activity by inducing production of IL-33 upon engagement by sialylated Fc IgGs. IL-33 production, in turn, led to release of IL-4 by basophils, which ultimately induced increased expression of FcγRIIb on effector myeloid cells (30). Expression of the inhibitory FcγRIIb modulates the threshold of activation of inflammatory cells, with increased FcγRIIb expression leading to reduced proinflammatory cytokine production (59–61). In this way, IVIg administration (or increasing endogenous Fc sialylation) can resolve acute inflammatory responses (31, 32, 49). Studies revealing the mechanistic basis of the antiinflammatory activity of IVIg suggest that endogenous IgG sialylation levels may drive susceptibility to inflammatory diseases.

Adaptive immunity

Aside from modulating inflammatory responses, Fc glycosylation has a separate function in regulation of adaptive immune responses. Antibodies that complex with vaccine antigens alter the way antigens are processed and can thus modulate the ultimate B and T cell responses to vaccination. The role of sialylated immune complexes in B cell selection was recently discovered in work drawing on the observation that sialylation of anti–influenza hemagglutinin IgG1 increases in the days following seasonal influenza vaccination in humans (29, 62). This work observed that enhanced sialylation correlated with increased antibody affinity after vaccination, implicating sialylated immune complexes within the germinal center in modulation of B cell selection after vaccination.

Selection of B cells based on affinity of the B cell receptor (BCR) is achieved, in part, by signaling through the inhibitory FcγRIIb on B cells, which acts to counterbalance the activating signal transduced through the BCR bound by antigen. In the absence of inhibitory FcγRIIb or with low-level expression or signaling, B cells lack appropriate activation thresholds and produce higher-titer, low-avidity IgGs (63, 64). Insufficient FcγRIIb signaling can lead to failure of immune tolerance, with autoantibody production in FcγRIIb$^{-/-}$ mice and low levels of B cell FcγRIIb found in patients with autoantibody-mediated diseases (64–71). In contrast, antigens presented in the form of immune complexes within germinal centers can engage the BCR but also FcγRIIb, thus elevating the threshold of BCR signaling required for activation of the B cell, which ultimately drives higher-affinity B cell responses (68, 72–74).

The mechanism by which sialylated IgGs impact B cell selection relies, at least in part, on the BCR-FcγRIIb axis. Sialylated immune complexes drive increased expression of FcγRIIb on B cells in a CD23-dependent manner. Increased FcγRIIb expression, in turn, elevates the threshold of BCR signaling required for cellular activation (Figure 2). Thus, immunization with sialylated hemagglutinin (HA) immune complexes results in higher-avidity IgG responses over immunization with asialylated HA immune complexes in CD23-expressing mice (29, 75). These experiments revealed a molecular mechanism whereby sialylated immune complexes can regulate the affinity of B cell responses after vaccination and also defined basal antibody sialylation as a driver of heterogeneity in the quality of vaccine responses. An important topic for future studies is whether the CD23 pathway can be targeted to improve the quality of antibody responses elicited additional vaccines. This could be especially useful in settings where avidity of the antibody response is a known determinant of vaccine efficacy, as with polysaccharide-based vaccines.

Heterogeneity and regulation of Fc domain glycosylation

A fascinating observation that has emerged from studies of human IgG repertoires in recent years is the considerable heterogeneity that exists in Fc glycosylation. Because antibody fucosylation and sialylation are central determinants of inflammatory thresholds, differences in these Fc saccharides among people are likely a key driver of distinctions in basal susceptibilities to inflammatory diseases (76–81).

Basal IgG1 Fc sialylation can differ by approximately 30% among people; this amount of variation is well within a range that modulates type II FcγR–mediated effector functions (29, 75, 81, 82). IgG1 Fc fucosylation varies by more than 20% among adults, with a majority of healthy adults having more than 90% fucosylation. Intriguingly, individuals appear to have set points

for Fc sialylation and fucosylation. This is observed in vaccination studies in which serial samples are taken over several weeks after vaccination. These studies show that even following large vaccine-elicited changes in Fc glycosylation, there is ultimately a general restoration of the abundance of various glycoforms found on prevaccination IgGs. Thus, people with relatively low sialylation on prevaccination IgGs, for example, can achieve average or high levels during vaccine-induced modulations, but ultimately return to relatively low Fc sialylation (29, 62). Why IgG Fc glycosylation differs among individuals is a topic of great interest. With respect to sialylation, recent work has shown that B cell production of the glycosyltransferase ST6GAL1 and sialylated antibody can be modulated by levels of estrogen or by IL-23 in vivo, suggesting that basal distinctions in hormones and cytokines may contribute to diversity in Fc sialylation (81, 83). Other general associations have been made between the abundance of different Fc glycoforms and age, sex, pregnancy, and treatment with high-dose IVIg (77, 82, 84, 85). The cumulative evidence suggests that both heritable and nonheritable factors likely influence set points for Fc sialylation.

Aside from differences in basal Fc sialylation and fucosylation among individuals, another level of heterogeneity is apparent within IgG repertoires of individuals. For example, differences in Fc glycoforms are associated with IgG Fab specificity. The anti-HA IgG response is one setting where the abundance of sialylation and fucosylation has been observed to differ depending on specificity of the Fab domain; anti-HA globular head IgGs are significantly more sialylated and fucosylated than are IgGs that react with the HA stem domain (29). In addition to the anti-HA response, other examples of Fc glycosylation segregating on Fab specificity are observed in autoimmune diseases such as granulomatosis with polyangiitis (GPA) and rheumatoid arthritis (RA). In these diseases, anti-PR3 (GPA) and anti-ACPA (RA) are found, and these antibody specificities have reduced Fc sialylation over total IgG sialylation from the same individuals (6, 78, 86). That the abundance of IgG Fc sialylation and fucosylation can be linked to antigen or domain specificity may be a consequence of differential glycosylation based on B cell subset. For example, ST6GAL1 and FUT8, the glycosyltransferases responsible for Fc sialylation and fucosylation, respectively, are expressed at higher levels in plasmablasts following influenza vaccination compared with memory or naive B cells (29).

With improved understanding of mechanisms regulating IgG Fc glycosylation, an important goal will be to harness specific pathways for a variety of therapeutic applications. For example, endogenous Fc sialylation could be modulated to treat diseases responsive to IVIg therapy, or vaccination strategies could be designed that would elicit antibodies with optimized Fc fucosylation to improve the effector function of vaccine-elicited antibodies and improve overall vaccine effectiveness.

Future directions

IgG Fc glycosylation is a mechanism for regulating Fc structure, and as a consequence it also regulates FcγR signaling and cellular effector functions. Numerous recent studies have revealed an unexpected degree of diversity in the Fc domain that translates into functional diversification in vivo. These studies have culminated in the understanding that variability among humans in Fc domain structural repertoires is likely a central driver of heterogeneity in human immunity (6, 29, 50–53, 56, 62, 78, 79, 81, 87). Rather than a static, monomorphic domain, the IgG Fc has evolved the capacity to modulate its structure and thus its binding interactions with families of Fc receptors, conferring a diversity of functional responses that can be associated with unique antigen-binding domains. While the role of sialylation and fucosylation in modulating Fc domain structure is now appreciated, there are considerable gaps in our knowledge about the mechanisms that drive Fc diversification and the contributions of Fc glycan modifications to the activity of non-IgG1 subclasses. Further, all IgG subclasses are differentially glycosylated among humans, and the consequences of this are largely unknown.

In addition to the effect of Fc glycoforms on non-IgG1 subclasses, an area where basic discovery studies are needed is in definition of receptors required for IgG function. There almost certainly exist FcγRs and cellular activities integral to IgG effector responses that remain to be identified. Vaccination is yet another critical topic for discovery. As effectiveness of vaccine responses generally depends on the Fab repertoire and the effector capacity of vaccine-elicited IgGs, the development of methods to elicit IgGs with specific Fc domain repertoires during vaccination could be transformative for the prevention and treatment of infectious diseases and could substantially advance antitumor vaccination strategies. Future studies will undoubtedly continue to shed light on how Fc domain repertoires impact susceptibility to human diseases and how FcγR pathways can be harnessed for therapeutic purposes.

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