Chapter 18
Glycosylation of Antigen-Specific Antibodies: Perspectives on Immunoglobulin G Glycosylation in Vaccination and Immunotherapy

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Contents

Abstract Exciting developments have been made in understanding antibody-mediated immunity, deepening understanding of antibody effector functions increasingly recognized as critical mechanisms of action beyond antigen recognition, and significantly broadening the evidence base for the importance of these effector mechanisms across diverse infectious and autoimmune diseases. Because these activities critically depend on the specific glycoforms present on a conserved site of the IgG Fc domain, relationships between the Fc glycosylation profiles of antigen-specific antibody pools and outcomes in infectious and autoimmune disease have

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begun to be defined, pointing to the key role of this posttranslational modification as a biomarker and mechanistic modifier of antibody-mediated immunity. Here we summarize studies evaluating the profiles and activities of antigen-specific antibodies elicited by infection and vaccination as well as within the context of allo- and autoimmunity, and consider current approaches to rational modification of Fc glycans in vivo.

Keywords Immunoglobulin · Antibody · Fc domain · Glycosylation · Vaccine · Allergy · Autoimmunity · IgG · Effector function

Abbreviations

ACPA	Anti-citrullinated protein antibodies
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ADE	Antibody-dependent enhancement
AMI	Antibody-mediated immunity
ASA	Antigen-specific antibody
CDC	Complement-dependent cytotoxicity
COVID-19	Coronavirus disease 2019
CSR	Class switch recombination
DC	Dendritic cell
DENV	Dengue virus
EndoS	Endoglycosidase S
Fab	Fragment antigen-binding
Fc	Fragment crystallizable
FcR	Fc receptor
FNAIT	Fetal or neonatal allo-immune thrombocytopenia
Fv	Fragment variable
GlcNAc	N-acetylglucosamine
HPA-1a	Human platelet antigen 1a
IdeS	IgG digesting enzyme S
IgG	Immunoglobulin G
IVIg	Intravenous immunoglobulin
K	Kell
mAb	Monoclonal antibody
MAC	Membrane attack complex
MBL	Mannose-binding lectin
MHC	Major histocompatibility complex
NK	Natural killer
HIV	Human immunodeficiency virus
RA	Rheumatoid arthritis
RhD	Rhesus D

RSV	Respiratory syncytial virus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

18.1 Introduction

Evidence of the critical importance of effector functions to antibody-mediated immunity (AMI) has been accumulating across studies of diverse infectious and autoimmune diseases. The ability to study and clinically leverage AMI was made a much simpler task after Kohler and Milstein's (Köhler and Milstein 1975) discovery of an approach to generate consistent, reliable, and reproducible monoclonal antibody (mAb) preparations, which represented challenges to the use of polyclonal sera prevalent at that time. Advanced molecular methods in antibody cloning (Wang et al. 2019; Hunter et al. 2019; Kim et al. 2014; Winzeler and Wang 2013; Chon and Zarbis-Papastoitsis 2011) and engineering (Bruggeman et al. 2018; Dekkers et al. 2018; Crooks et al. 2018; Dekkers et al. 2016) have complemented the discovery of diverse Fc receptors (FcR) (Bournazos and Ravetch 2017; Castro-Dopico and Clatworthy 2016; Wu et al. 2014; Hirvinen et al. 2013; Nimmerjahn and Ravetch 2008a; Lazar et al. 2006; Hogarth 2002) and development of elegant knockout mouse models (Walsh et al. 2016; Verkoczy 2017; Stackowicz et al. 2020) to enable further basic science exploration and to support therapeutic optimization of AMI. In parallel, higher resolution means of profiling serum antibodies have accompanied these advances and greatly expanded the ability to interrogate polyclonal responses in serum and tissue. The high throughput of many of these profiling approaches has now turned the heterogeneity observed among polyclonal samples into a strength, providing means to interrogate the features and activities of antibodies that are associated with AMI.

18.2 Antibody Effector Functions

Antibodies play an important role in both effecting and regulating an immune response. They have the capacity to either amplify or dampen an inflammatory immune response based on their specificity, affinity, titer, isotype, and glycosylation profile. While the antigen-binding fragment (Fab) domain confers antigen specificity, the crystallizable fragment (Fc) domain is responsible for linking antigen recognition to downstream effector functions (Schroeder and Cavacini 2010). Antibodies can neutralize pathogens by directly binding through the Fab domain and occluding the binding of the pathogen or its toxins to cognate receptors. Such Fab-mediated antibody action is complemented by Fc domain engagement of complement proteins and FcR (Fig. 18.1). There are broadly two categories of FcRs—activating and inhibitory—that are ubiquitously expressed on human hematopoietic

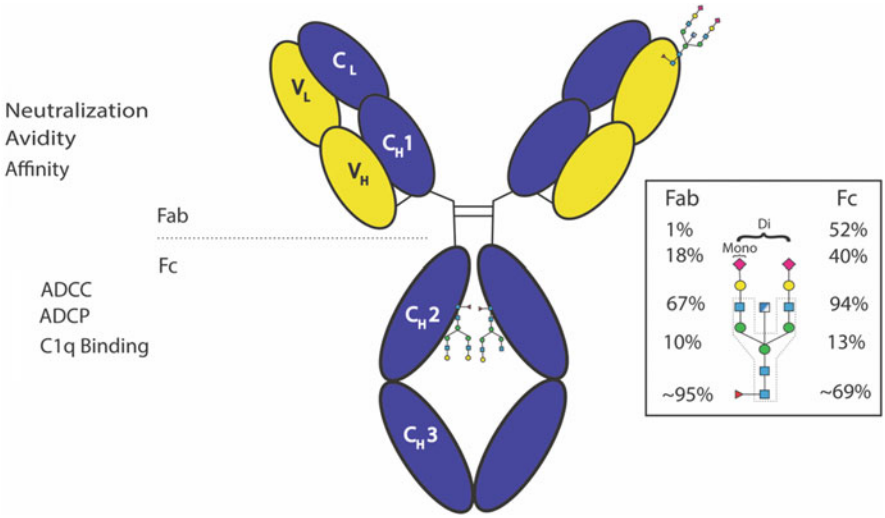

Fig. 18.1 Antibody glycosylation sites in IgG. Glycosylation sites can be present in the Fab region (15–25% of IgGs), but are always present in the Fc region. There are distinct qualitative differences among the glycans commonly found at these sites, however, the importance of the glycans in mediating function via respective antibody domains is a shared characteristic. These glycans share the core heptasaccharide (dotted line in the inset) to which extensions of specific sugars are attached. Fc glycans tend to be heavily fucosylated whereas the Fab glycans have generally been observed to exhibit relatively high levels of sialylation. Adapted from Bondt et al. (2014)

cells and play a key role in orchestrating potent antibody-mediated effector immune responses in the context of both protective immunity to pathogens and pathogenic immune responses to self (Fig. 18.2).

The most widely studied Fc-mediated antibody effector functions are antibody-dependent cell-mediated cytotoxicity (ADCC) (Worley et al. 2018), antibody-dependent cellular phagocytosis (ADCP) (Gerber and Mosser 2001), and complement-dependent cytotoxicity (CDC) (Goldberg and Ackerman 2020). These activities are induced by engagement of the FcγRs on innate effector cells, or by soluble complement cascade initiators, such as C1q or Mannose Binding Lectin (MBL), by the Fc domain of antibodies that are bound to a target antigen. ADCC is characterized by FcγR engagement that causes the release of cytotoxic granules that contain perforin and granzyme, resulting in the killing of target cells (Smyth et al. 2005). FcγRIIIA-expressing Natural Killer (NK) cells are widely considered to be an important contributor to ADCC and are often assayed in vitro. However, in vivo, neutrophils, monocytes, and macrophages are also capable of driving ADCC and have been found to make important contributions to antibody mechanism of action (Smyth et al. 2005; van Erp et al. 2019).

ADCP or opsonophagocytosis is the uptake of immune complexes or antibody-coated antigens by phagocytic cells including monocytes, macrophages, dendritic cells (DCs), and others that express FcγRI, FcγRII, and/or FcαRI, each of which can

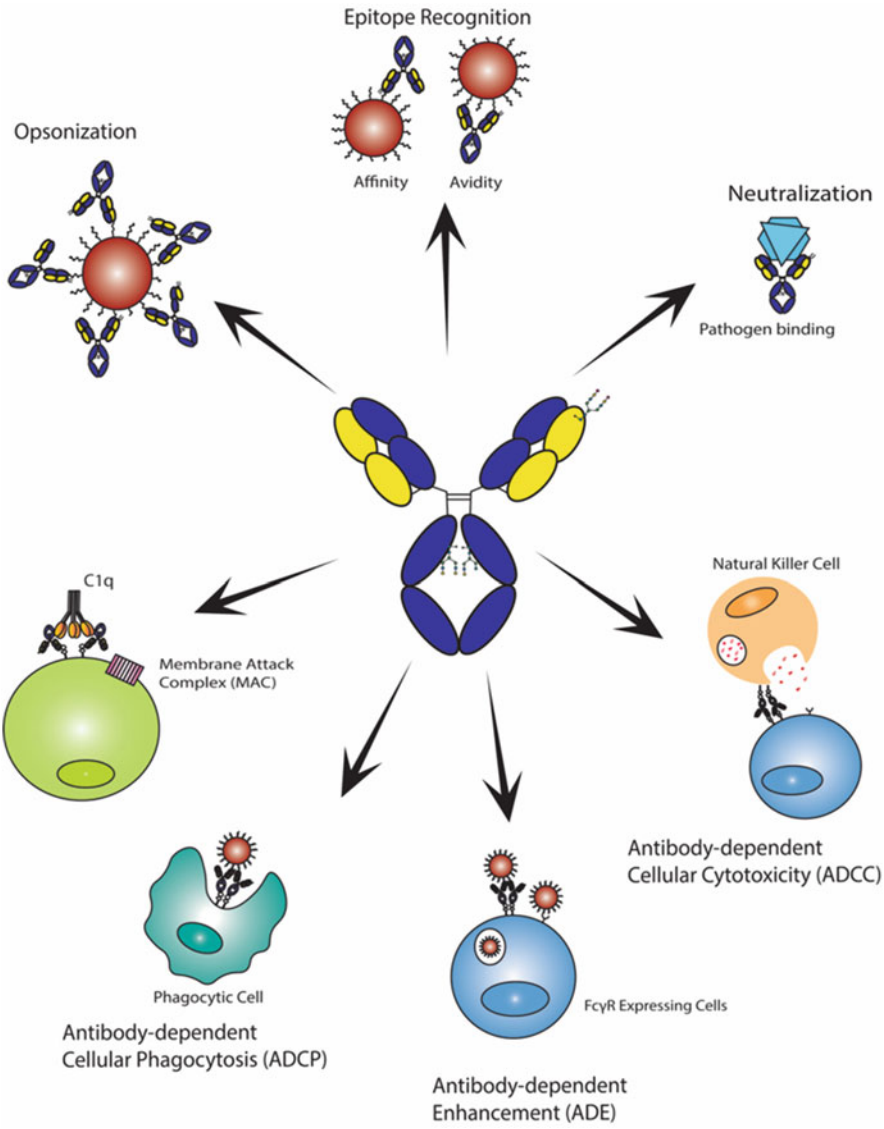

Fig. 18.2 Antibody mechanisms of action. While the Fab domain functions are directly driven by antigen recognition, Fc-mediated functions result from recruitment of various components of the innate immune system

mediate immune complex uptake (Li and Kimberly 2014). ADCP mediates clearance of immune complexes by trafficking them to the lysosomes for degradation and antigen processing for presentation on Major Histocompatibility Complex (MHC) molecules on the cell surface (Mantegazza et al. 2013). Previous work on influenza virus has shown that ADCP contributes to protection from infection in mice (Huber

et al. 2001; He et al. 2017) and potentially plays a role in recovery from severe infections in humans (Vanderven et al. 2017; Ana-Sosa-Batiz et al. 2016). Associations between ADCP and improved outcomes in other disease settings such as HIV (Barouch et al. 2013, 2015), West Nile Virus (Vogt et al. 2011) in humans, and Respiratory Syncytial Virus (RSV) (Bukreyev et al. 2012) have also been recently established.

Besides ADCC and ADCP, antibodies can also induce complement activation. The complement cascade contributes to pathogen elimination either directly, by means of complement-dependent cytotoxicity (CDC), or indirectly, through phagocytic clearance of complement-coated targets and the induction of an inflammatory response (Goldberg and Ackerman 2020; van Erp et al. 2019; Grafals and Thurman 2019; Casadevall and Pirofski 2012). The complement cascade consists of a large number of distinct plasma proteins that react with one another to opsonize pathogens, inducing a series of inflammatory responses that help to fight infection (Noris and Remuzzi 2013). A number of complement proteins are proteases that are themselves activated by proteolytic cleavage (Dunkelberger and Song 2010). The terminal complement components assemble into the membrane attack complex (MAC), resulting in lysis of the pathogen-infected cell. Complement has been shown to have both protective and pathogenic effects in various disease conditions. In HIV (Barouch et al. 2013, 2015; Pittala et al. 2019), influenza (Co et al. 2014; Wu et al. 2015), and vaccinia (Benhnia et al. 2009) infection, antibody-mediated CDC has been shown to correlate or mechanistically contribute to antibody antiviral activity. Alternatively, complement-mediated activation has also been associated with disease severity (Nascimento et al. 2009; Churdboonchart et al. 1983; Füst et al. 1994). Lastly, the binding of complement-coated immune complexes to complement receptor 2 on B cells is reported to lower the B cell activation threshold, thereby promoting long-lived adaptive immunity and higher antibody levels (van Erp et al. 2019; Hebell et al. 1991; Gonzalez et al. 2010).

18.3 Immunomodulatory Antibody Activities

In contrast, anti-inflammatory effects of antibodies can help in alleviating severe immune damage. Based on this concept, administering intravenous immunoglobulin (IVIg) to treat inflammatory conditions such as autoimmune disease has found an important clinical application (Bayry 2016). While the underpinnings of IVIg mechanisms have yet to be clearly elucidated (Schwab and Nimmerjahn 2013), different mechanisms of action such as neonatal Fc receptor blockade resulting in accelerated clearance of autoantibodies (Li and Kimberly 2014), direct interaction with the inhibitory FcγRIIb (Nagelkerke and Kuijpers 2015), or occlusion of activating receptors and tempering the inflammatory effector responses (Nimmerjahn and Ravetch 2008b) have been proposed. However, since IVIg treatment is used to treat various diseases, it is likely that the mode of action differs per clinical setting.

18.4 Induction and Regulation of Antigen-Specific Antibodies

During an immune response, B cells are stimulated to mature and to undergo class switch recombination (CSR) resulting in genetic modification of the IgH locus and selection of the antibody isotype and subclass to be secreted (Stavnezer and Schrader 2014). Just as B cells undergo rounds of somatic hypermutation over the course of affinity maturation as they migrate in and out of regions in the germinal center, CSR can occur in rounds with repeated switching to downstream types (Mesin et al. 2016). This heterogeneity in the amino acid sequence of both variable fragment (Fv) and Fc regions is coupled to further functional diversification via incorporation of one of the >30 possible glycoforms (Jennewein and Alter 2017) in the conserved N-linked glycosylation motif. While multiple isotypes are glycosylated in the Fc, we will focus on glycosylation in the context of the four IgG subclasses (Vidarsson et al. 2014).

In the past 20 years, the role of antibody glycosylation as an important parameter modulating the potency of effector functions has been firmly established through advances in monoclonal antibody research and development, as well as in studies of natural immune responses in the context of infectious and autoimmune disease. Here we focus on recent research considering the glycosylation of antigen-specific antibodies in these settings.

18.5 Importance of Ab Glycosylation

The Fc domain contains a consensus N-linked glycosylation site that is typically occupied by a heptasaccharide core structure consisting of four N-acetylglucosamine (GlcNAc) and three mannose moieties that form a biantennary complex (Liu 2015). Additional glycosylation features such as fucose, galactose, sialic acid, and GlcNAc can be added later to the core structure to produce over 30 distinct glycovariants. As both heavy chains are glycosylated, a single IgG molecule can have a diverse array of glycosylation heterogeneity (Jefferis 2009). Nuclear magnetic resonance (NMR) studies have shown that variability in the glycans at this conserved position has a profound effect on the hinge region conformation (Yamaguchi et al. 2006). Similarly, interactions with Fcγ and other IgG and glycan receptors are entirely dependent on or modified by glycan composition and conformation, thus the type of glycan occupying this site modifies antibody effector function (Saunders and Conceptual 2019). Unlike genetically templated factors that impact IgG activity, such as Fv sequence and Fc subclass, antibody glycosylation is remarkably varied, resulting in a high level of microheterogeneity that facilitates the fine tuning of antibody function (Alter et al. 2018a). These dynamic changes in antibody glycosylation can have a subtle or profound effect in their interactions or downstream functions.

18.6 Typical Serum IgG Fc Glycan Composition

Given the importance of IgG Fc glycans, the composition of serum antibodies has been evaluated in a number of populations, providing insight into changes associated with age, sex, hormone levels, and disease status. Nonetheless, "typical" compositions have been articulated among healthy individuals (Fig. 18.1), and deviations from this profile suggest active processes regulating this posttranslational modification at multiple levels.

Serum IgG Fc is typically overwhelmingly fucosylated (>90%) (Gudelj et al. 2018). However, skewed glycosylation variants, produced by chemoenzymatic modifications or expressed in engineered cells, have been produced that lack this fucose moiety, and as a result exhibit significantly improved effector function. For example, an afucosylated form of an anti-CD20 IgG1 showed a 50-fold improvement in binding to FcγRIIIa and enhanced ADCC activity (Shields et al. 2002). Later, structural studies found that the fucose on the Fc glycan clashes with a $GlcNAc_2$ group of an FcγRIIIa glycan, thereby providing a structural rationale to the improved ADCC activity of afucosylated antibody (Ferrara et al. 2011).

About 10% of all circulating IgGs in healthy human adults exhibit bisected Fc glycans (Gudelj et al. 2018), which have been shown previously to relate to ADCC activity (Hodoniczky et al. 2005). However, this amplification in ADCC, caused by the increased engagement of the FcγRIII, is believed predominantly to be due to the indirect role of bisection in decreasing fucosylation, rather than a direct consequence of its presence in the antibody structure (Shinkawa et al. 2002).

Similarly, agalactosylated, monogalactosylated, and digalactosylated glycan structures account for approximately 35%, 35%, and 15% of circulating IgG Fc-glycans, respectively (Gudelj et al. 2018). A prominent bias towards agalactosylated antibodies has been observed in people with active autoimmune and inflammatory diseases (Parekh et al. 1989; Tomana et al. 1992; Rademacher et al. 1994; Decker et al. 2016), however, a clear consensus on cause or consequence is yet to be achieved (Alter et al. 2018a). Furthermore, there are conflicting reports on the role of galactosylated antibodies in mediating proinflammatory activities, with some reports observing the presence of galactosylation on the IgGs to enhance the ADCC and complement binding (C1q) in vitro (Nimmerjahn et al. 2007; Peschke et al. 2017; Thomann et al. 2015; Tsuchiya et al. 1989), while others have noted a dampening of an inflammatory response by highly galactosylated immune complexes (Karsten et al. 2012). A lack of correlation between the presence or absence of galactosylation on IgGs and corresponding in vivo activity has also been reported (Nimmerjahn et al. 2007), suggesting that the consequences of variable galactosylation may be best investigated per disease model and per antibody.

Lastly, approximately 10% of circulating IgG Fc is sialylated (Gudelj et al. 2018). Sialylated IgG Fc is associated with an anti-inflammatory profile of antibodies in mouse models, in which neuraminidase-treated, asialylated pooled human IgG (IVIg) has been observed to abrogate the normally anti-inflammatory activity of IVIg (Kaneko et al. 2006). However, this mechanism of action remains controversial

in humans. Discrepant observations have been made as to the ability of IgG to interact with the candidate receptor proposed on the basis of mouse studies (Anthony et al. 2008; Temming et al. 2019), and sialylated IgG has shown slightly elevated binding to activating FcγR and C1q, and associated effector functions (Dekkers et al. 2017; Subedi and Barb 2016), which would suggest a greater inflammatory capacity.

18.7 Variations in Ab Glycoprofiles

Deviations from these "typical" profiles have been associated with diverse physiological and immunological states. For example, changes in total serum IgG Fc glycosylation are observed in early life (Cheng et al. 2019), in adolescence (Gudelj et al. 2018; de Haan et al. 2016), and in association with hormonal status (Ercan et al. 2017), as well as more gradual changes during immune senescence (Krištić et al. 2013), across a broad range of glycoforms and constituent sugar moieties. In the context of ongoing inflammation, such as observed in chronic infection (Moore et al. 2005) or autoimmunity (Parekh et al. 1985), global IgG Fc glycosylation is often modified, showing reduced galactose and sialic acid content (Lastra et al. 2009).

Beyond approaches to evaluate these global changes, the role of Fc glycans in antibody function has also motivated the development of robust methods to define the glycosylation profiles of antigen-specific antibodies (ASA) purified from serum. Early questions about ASA fractions related to whether they are typically composed of IgG Fc glycovariants with similar prevalence to those observed for total serum IgG, and if not, whether glycoprofiles vary by pathogen, antigen, and epitope specificity.

18.8 ASA Glycosylation in Infectious Disease

In the context of responses to the HIV envelope protein among chronically infected individuals, HIV envelope glycoprotein-specific antibodies were found to exhibit reduced galactosylation, fucosylation, and sialylation (Ackerman et al. 2013), even when compared to global serum IgG Fc glycan profiles that were shifted in these same directions as compared to uninfected and acutely infected individuals (Moore et al. 2005). Among ASA, galactosylation levels correlated with Ab-dependent inhibition of viral infection and replication and were consistent with glycosyltransferase and glycosidase expression in peripheral B cells (Ackerman et al. 2013). Perhaps surprisingly, these global and HIV-specific plasma IgG Fc glycan changes were not resolved by either antiretroviral drug therapy or in the context of spontaneous virus control. Subsequent studies have shown the contribution of HIV-specific IgG glycans to predicting HIV-specific antibody effector functions (Alter et al. 2018b) and vaccine efficacy (Vaccari et al. 2016; Ackerman et al. 2018).

These and other early studies have firmly established that ASA can differ from total serum IgG in their glycosylation states. As methods for analysis of ASA have advanced, analysis of ASA targeting different proteins has become increasingly feasible but not yet common. To the extent studies have addressed multiple target antigens, there has been some evidence for consistent glycoforms across distinct specificities and other cases in which different antigen-specificities, or even different epitope-specificities within the same protein have shown distinct profiles. For example, in tuberculosis, distinct ASA IgG Fc glycan profiles for two different antigen types were reported to show similar glycan profiles to each other, but with striking decreases in fucose and increases in galactose, sialic acid, and bisecting GlcNac as compared to total serum IgG Fc (Lu et al. 2020). In contrast, Wang et al. reported that the abundance of sialylation and fucosylation among influenza hemagglutinin-specific (HA) IgG differed depending on specificity of the Fab domain. Antibodies to the HA globular head were significantly more sialylated and fucosylated than those directed against the HA stem domain (Wang and Ravetch 2019), though it may be important to keep in mind that the globular head functions as a sialic acid-binding protein.

One of the most interesting examples of the effect of ASA Fc glycosylation comes from the setting of flavivirus infection. This family of viruses has been associated with a phenomenon called Antibody-Dependent Enhancement (ADE), in which virus-specific antibodies increase infection of FcγR-bearing target cells. Among these, dengue is a mosquito-borne pathogen caused by four distinct but closely related dengue virus (DENV) types. Recovery from infection is believed to typically provide immunity against infection from the same type. However, cross-type immunity is partial and temporary. Subsequent (secondary) infection by another serotype is associated with an increased risk of developing severe dengue via ADE (Katzelnick et al. 2017; Guzman et al. 2013). While prior work has shown that waning antibody titer is associated with severe disease upon secondary exposure (Katzelnick et al. 2017), recent work has highlighted the potential importance and clinical impact of the glycosylation of dengue-specific antibodies. As perhaps the most elegant setting in which to evaluate ADE, severe disease of neonates is associated with the level of passively transferred maternal dengue-specific antibody that is afucosylated, resulting in dengue hemorrhagic fever or dengue shock syndrome (Wang et al. 2017; Thulin et al. 2020; Khandia et al. 2018). This potent ADE response is thought to manifest via non-neutralizing, dengue-specific antibodies that exhibit increased affinity to the activating FcγRIIIA receptor.

In the context of coronavirus disease 2019 (COVID-19), Fc glycans of IgG antibodies to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) envelope spike and nucleocapsid proteins differ from those of total serum IgG (Larsen et al. 2020). In this study, these profiles were observed to differ between spike and capsid, and multiple studies have observed that spike-specific IgG Fc afucosylation is correlated to disease severity (Larsen et al. 2020; Chakraborty et al. 2020), with some evidence that they may contribute to pathology via inducing inflammatory responses from macrophages (Hoepel et al. 2020). Global serum IgG glycans have also been reported to diverge according to COVID-19 severity, with

decreased bisecting GlcNAc observed in multiple cohorts (Petrovic et al. 2020). The role of IgG Fc glycosylation of ASA remains to be defined in many more infectious disease settings. Like in COVID-19, HIV, and other settings in which ASAs have been profiled, intriguing observations regarding differences in global IgG Fc glycosylation abound—such as in meningococcal sepsis (Haan 2018), visceral leishmaniasis (Haan 2018; Gardinassi et al. 2014), and tuberculosis (Lu et al. 2016, 2020)—and have been found to relate to disease status or outcomes.

18.9 ASA Glycosylation in Allo/Autoimmunity

Rheumatoid arthritis (RA) is a common systemic inflammatory autoimmune disease in which joint synovium is affected by a dysregulated immune system. RA is typically associated with serological evidence of systemic autoimmunity as indicated by the presence of autoantibodies in serum and synovial fluid (Coutant 2019; Song and Kang 2010). Instead of being characterized by specific reactivity to a particular autoantigen, RA is associated with antibodies reactive against a wide spectrum of autoantigens, which can make the etiology of disease progression in RA patients very different. Among various autoantigens targeted in RA, anti-citrullinated protein antibodies (ACPA) have been identified as a useful marker in diagnosis (Coutant 2019) and predicting whether undifferentiated arthritis will progress to RA (Forslind et al. 2004). ACPA are associated with an increased risk of developing bone erosions (Rönnelid et al. 2005; Rycke et al. 2004), suggesting their potential to contribute to joint pathology. Like total serum IgG, long known to show decreased galactosylation, ACPA are observed to exhibit further reduction in sialylation and galactosylation (Scherer et al. 2010; Ohmi et al. 2016), though there is some evidence that the IgG subclasses may differ from each other in this regard (Lundström et al. 2014). Reinforcing the controversy regarding the potentially conflicting roles of sialylated IgG in different species, but supporting the role of glycoengineered ASA as therapeutic interventions, sialylated ACPA have been shown to reduce arthritis pathology in a mouse model (Ohmi et al. 2016).

Whether ACPA are a cause or consequence of RA status remains controversial, but they have been reported to activate effector cells via FcγR (Clavel et al. 2008), whose allotypic and copy number variation have sometimes but not always been observed to associate with RA status and severity (Thabet et al. 2009; Kastbom and Ahmadi 2005; Nieto et al. 2000; Radstake et al. 2003). Further, several longitudinal studies have observed that galactosylation and sialylation levels of ACPAs decreased shortly before symptom onset in patients who had ACPA but no evidence of RA at baseline (Pfeifle et al. 2017; Harre et al. 2015; Rombouts et al. 2015), suggesting the potential value of measuring the level of ACPA galactosylation/sialylation as a biomarker to predict the risk of progression from pre-clinical disease to chronic inflammatory disease. Beyond differences between ACPA and total IgG Fc glycosylation, differences in ACPA Fc glycan profiles have also been noted between individuals with and without rheumatoid factor, and between serum and

synovial fluid (Scherer et al. 2010). While some have interpreted these differences to potentially relate to active alteration of Fc glycans in affected joints, the lack of differences in total serum and synovial fluid IgG1 agalactosylation suggests that alternative mechanisms may be at play. To this end, ACPA-secreting plasma cells have been reported to exist in synovial fluid (Rodríguez-Bayona et al. 2007), suggesting the possibility that differences in systemic versus synovial ACPA Fc glycosylation may be driven by differences associated with plasma cells in the synovium and elsewhere.

Beyond these alterations in Fc glycosylation, ACPA have more recently been reported to exhibit striking glycosylation of their Fab domains. Unlike total IgG, a majority of ACPA variable domains are glycosylated (Lloyd et al. 2018; Hafkenscheid et al. 2019; Hafkenscheid et al. 2017). Unlike their Fc domains, these APCA Fab glycans are overwhelmingly sialylated (Hafkenscheid et al. 2017). Variable domain glycosylation has also been reported to modify antigen binding among ACPA (Rombouts et al. 2016), suggesting the potential for antibody glycosylation in both variable and crystallizable domains to contribute to RA pathogenesis.

Functional consequences of variations in the profile of ASA have also been reported in fetal or neonatal allo-immune thrombocytopenia (FNAIT). In this disease condition, fetal allo-antigens induce production of maternal antibodies that are then transported across the placenta and drive lysis of fetal cells. While allotypic variation of a variety of maternal fetal antigens is possible, the best studied is that of rhesus D (RhD) antigen incompatibility. Curiously, this incompatibility, which resulted in hemolytic disease in 1% of babies born through the 1940s, 40% of which would die as a result (Bowman 2003), is treated by administration of IVIG from RhD-sensitized donors. While like IVIG used in other indications, the precise mechanisms of this intervention remain unclear; prevention of sensitization, immunomodulatory effects, and accelerated clearance of endogenous maternal IgG have all been proposed as candidate mediators. To this end, the RhD-specific antibodies in at least one commercial product show increased galactosylation and sialylation relative to the entire mixture of antibodies in that product (Winkler et al. 2013), suggesting their potential immunosuppressive character. Evaluations of the mechanism of action have been hampered by the difficulty in recapitulating protective effects of polyclonal RhD IgG with monoclonal antibodies. The difference in the effect of polyclonal versus monoclonal antibody infusions may relate to differential glycosylation of RhD-specific fraction or entire pool, differences in affinity and avidity, altered red blood cell clearance capacity, or other factors, but have led to observations of alternatively enhanced or inhibited maternal sensitization, leaving many unanswered questions (Kumpel 2007; Kumpel et al. 1995). To this end, it has been recently reported that RhD-specific monoclonal antibodies varied in their ability to clear RhD+ target cells and prevent alloimmunization, dependent on their fucosylation status and associated ADCC activity (Kumpel et al. 2020). Similarly, in the context of seropositive mothers, IgG Fc fucosylation of RhD-specific antibodies have been found to correlate with ADCC activity and low fetal neonatal hemoglobin levels (Kapur et al. 2014a).

Despite questions as to mechanism, RhD+ serum IgG has all but eliminated pregnancy loss and neonatal death from RhD incompatibility in much of the world. In contrast, other less frequently observed incompatibilities have no effective preventative interventions. For a number of these antigens, maternal antibody titer is a poor indicator of pathology, and in some of these settings, variation in ASA-Fc glycosylation has been investigated for its predictive value. Here, more mixed results as to the importance of glycosylation profiles of fetal antigen-specific antibodies have been observed. As compared to RhD-specific antibodies, those recognizing red blood cell antigens K, c, and E were less distinct from total plasma IgG Fc glycans than those recognizing RhD, but nonetheless, afucosylation of Kell (K)-specific antibodies and high galactosylation and sialylation of anti-c antibodies were correlated with severe anemia of the fetus (Sonneveld et al. 2016a). In a small follow up study of maternal K-specific antibodies, IgG1 and IgG3 fractions were shown to exhibit similar glycoform prevalences, and while the previously observed relationship between afucosylation and disease severity did not meet an arbitrary significance threshold of $p = 0.05$, galactose content was shown to correlate with disease severity (Sonneveld et al. 2018).

Beyond red blood cell alloantigens, Fc glycoforms of human platelet antigen 1a (HPA-1a)-specific antibodies have been analyzed. Like other maternal alloantibody responses, HPA-1a-specific antibodies show markedly decreased levels of fucosylation as compared to total serum IgG1 (Kapur et al. 2014b). These significantly less fucosylated anti-HPA-1a antibodies showed enhanced phagocytosis of platelets on account of higher binding affinity to FcγRIIIa and FcγRIIIb, but not to FcγRIIa, compared with antibodies with a high amount of Fc fucose. Most critically, the extent of HPA-1a-specific antibody Fc fucosylation was shown to correlate with clinical disease severity. In a follow-up study, stability of ASA Fc glycans was defined and correlations between bleeding severity and fucose, galactose, and antibody titer were observed (Sonneveld et al. 2016b). Similarly, Jo1 anti-histidyl tRNA synthetase autoantibodies, which are observed in idiopathic inflammatory myopathy and anti-synthetase syndrome, have demonstrated similar reductions in galactose, sialic acid, and fucose, with glycoprofiles relating to disease status (Fernandes-Cerqueira et al. 2018).

Collectively, auto- and alloimmune responses have supported the importance of Fc glycans of ASA to diverse antigens. These observations have motivated investigation of deglycosylated IgGs to prevent FNAIT (Bakchoul et al. 2013), and sialylated ACPA to treat RA (Ohmi et al. 2016). While similar evaluation of alloantibodies in the setting of organ transplant has proven challenging, the role of effector functions is well established, with assessment of complement deposition associated with transplant- or donor-specific antibodies (DSA) forming part of the basis for evaluation of suitability of transplant (Zeevi et al. 2013; Mohan et al. 2012; Stegall et al. 2011; Lefaucheur et al. 2010), and enzymatic Fc restriction of serum IgG showing potential in reducing transplant loss associated with DSA positive organ recipients (Jordan et al. 2017).

18.10 In vivo Fc Glycan Programming

The importance of IgG Fc glycans to Ab biology in vivo has motivated a number of interventions that take advantage of this dependence. Beyond glycoengineering of therapeutic antibodies to optimize their activity, sophisticated new approaches are being explored to control antibody activity. These include leveraging B cell-independent sialylation (Jones et al. 2016) by administration of exogenous galactosyl and sialyltransferase in order to accomplish in vivo sialylation and thereby ameliorate autoimmune disease (Pagan et al. 2018). Similarly, changes in sialyltransferase expression induced by estrogen therapy suggest alternatives to exogenous enzyme therapy (Engdahl et al. 2018).

As opposed to extending IgG Fc glycans, glycan restriction is also being employed toward the same goal of reducing autoimmunity. Glycosidase therapy, most notably EndoS from *S. pyogenes*, the same organism that expresses the IgG protease IdeS used to disarm HLA alloantibodies in kidney transplant, has been investigated in diverse autoimmune conditions in animal models. These settings include IgG-driven thrombocytopenia purpura (Collin et al. 2008), collagen autoimmunity (Hirose et al. 2012), anti-neutrophil cytoplasmic autoantibody-mediated glomerulonephritis (van Timmeren et al. 2010), and autoimmune hemolysis (Allhorn et al. 2010). Challenges to clinical translation remain, including the consequences of globally eliminating effector function non-specifically, as well as the induction of anti-enzyme antibodies, but recent translation of the Fc protease IdeS suggests that these barriers may be surmountable (Collin and Bjorck 2017).

Other possibilities, such as the ability to vaccinate to drive specific inflammatory or anti-inflammatory antibody responses, also exist. A future in which allergen therapy leverages B cell transcriptional programs to not only undergo CSR toward less inflammatory IgG4 molecules but also toward anti-inflammatory glycans comes to mind, as has been shown to lessen allergic reactions in a mouse model using a recombinant glycoengineered antibody (Epp et al. 2017). To this end, Vestrheim et al. considered four distinct bacterial and viral vaccines and observed that the IgG subclass that dominated the response exhibited a temporal increase in galactosylation and sialylation for most vaccinees (Vestrheim et al. 2014). Other studies have observed this effect only within the ASA fraction (Selman et al. 2012).

With a more nuanced perspective, Larsen et al. compared and contrasted ASA targeting enveloped and non-enveloped viral pathogens and found decreased fucose content that is consistent with responses to infection by enveloped viruses, though to varying extents (Larsen et al. 2020). Natural infection, at least in the case of Hepatitis B Virus, was found to better induce afucosylated IgG1 as compared to immunization with a protein subunit vaccine. In contrast, attenuated Mumps virus vaccination induced a similar level of IgG1 afucosylation as natural infection. A study considering HIV-specific IgG Fc glycans observed that vaccination was able to overcome the normally observed variations in total serum IgG associated with geography (Mahan et al. 2016). ASA showed similar glycosylation patterns for a given vaccine, but distinct vaccine regimens resulted in distinct ASA glycosylation profiles.

These and complementary observations related to difference in induction of the IgG subclasses mediated by distinct antigen, pathogen, or vaccine stimuli suggest the existence of "rules" regulating the CSR and glycosylation processes in B cells. While refined insight into these pathways continues to develop, using an in vitro B-cell culture system resembling the in vivo T-cell-dependent antibody production, Wang et al. showed that B-cells secreted variably glycosylated IgG1 when stimulated with TLR ligands, metabolites, and cytokines (Wang et al. 2011). Indeed, because the antibody Fc domain itself can regulate responses by antigen-presenting cells and B-cells, manipulation of Fc glycans in the context of immune complex vaccines has been used to intentionally influence subsequent Ab induction/maturation (Lofano et al. 2018).

18.11 Summary and Future Perspectives

Distinctly different global IgG and ASA Fc profiles have been observed in both infectious disease and auto- and alloimmune settings. Studying the Fc glycosylation profile of ASA presents an excellent opportunity to understand the mechanistic underpinnings and the in vivo regulation of the diverse adaptive immune processes that define protective and pathological humoral responses. To this end, many unanswered questions remain.

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Compliance with Ethical Standards

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Conflict of Interest PB declares he has no conflict of interest. MEA declares that she has no conflict of interest.

Ethical Approval This chapter does not contain any studies with human participants or animals performed by any of the authors.

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