Trends in Immunology

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Review

The Immunoregulatory Roles of Antibody Glycosylation

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Beyond their role in neutralization, antibodies mediate functions such as phagocytosis, cytotoxicity, and maintenance of immune homeostasis. Two modifications to the constant domain control antibody activity: theirreversible genomic selection of isotype/subclass and alterations in glycosylation. Because glycosylation alters the affinity of antibodies for Fc receptors, evidence suggests that glycosylation is a central mechanism for the immune system to tune a broad range of biological activities. While monoclonal therapeutics have exploited glycosylation to improve function, its *in vivo* control and whether it may be selectively harnessed to target pathogens and/or tumors is unknown. Here, we review the process of antibody glycosylation, how it changes with disease, how it impacts antibody functionality, and the potential for deliberately controlling this biological activity.

Antibody Glycosylation Controls Antibody Activity

Antibodies represent the correlates of protection for nearly all licensed vaccines [1]. Though the mechanisms underlying this protection are unknown for most clinically approved vaccines, the ability of antibodies to neutralize pathogens or toxins is thought to be crucial [1]. However, additional activities including antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) are emerging as important contributors to protective immunity. They have been implicated in the destruction of opsonized pathogens and thus help mediate protection following vaccination and infection [1–5]. Similarly, in cancer and autoimmunity, antibodies have been exploited to block signaling pathways, and also to directly target and drive elimination of tumor cells [6–9]. Importantly, recent successes in monoclonal therapeutic design have been achieved through technological breakthroughs that facilitate the generation of modified antibodies that exhibit enhanced abilities to recruit innate immune killing through alterations in antibody Fc glycosylation [6]. The enhanced clinical potency of these Fc-modified therapeutics argue that antibody glycosylation represents a novel and important means to tailor and tune antibody functionality to direct immune control and clearance, and that a better understanding of the mechanisms that control the post-translational modification of the Fc domain of antibodies *in vivo* could improve the therapeutic efficacy of antibodies for monoclonal therapeutics and next-generation vaccine design.

Antibodies have a single N-linked glycan attached at asparagine 297 (Asn297) of each heavy chain (Figure 1) that has been suggested to impact antibody conformation via specific glycan–protein and glycan–glycan interactions [10]. The shape of the Fc region defines the capacity of the antibody to interact with innate immune Fc receptors, which direct antibody functionality. Humans have six classical Fc receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb), found in varied combinations on all innate immune cells at different expression levels (Box 1) [11]. Antibody Fc domains also interact with complement proteins (C1q and mannose-binding lectin), other C-type lectin receptors that drive effector functions through different

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Antibody glycosylation defines the functional potential of the antibody by delineating the structure of the antibody Fc region and determining which Fc receptors it can bind to in order to recruit effector cells.

The effector functions that antibodies mediate, including cytotoxicity and phagocytosis, are critical for protection against and prevention of many diseases.

Antibody glycosylation has been harnessed to improve the efficacy of monoclonal therapeutics.

Antibody glycosylation can be modulated by vaccination, indicating that rational immunogen design could seek to elicit a specific antibody glycosylation response.

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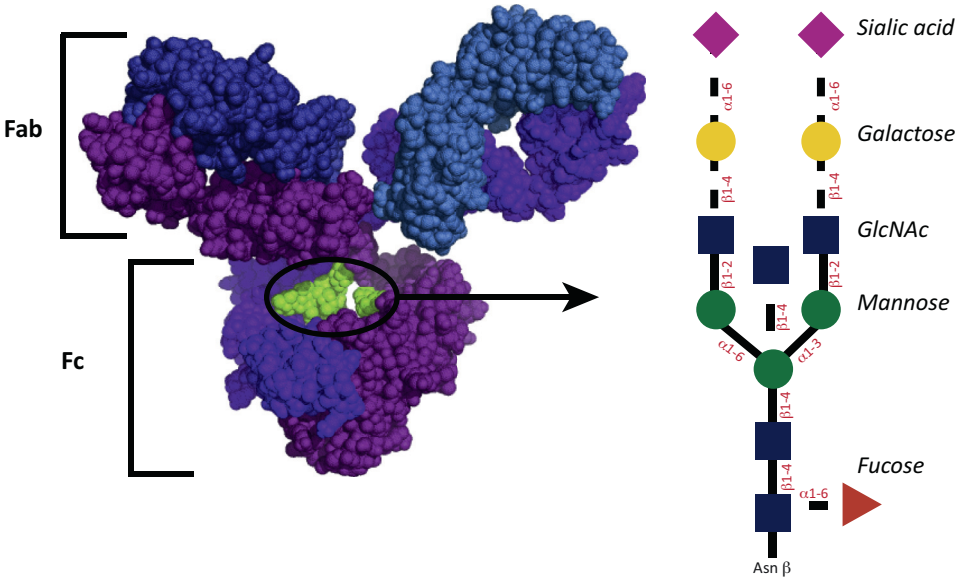

Figure 1. Antibody and Glycan Structure. IgG antibodies are composed of two heavy chains (purple) and two light chains (blue), which together form two functional domains:the antigen-binding (Fab) and crystallizable/constant domains (Fc). Each heavy chain Fc contains a glycan at Asn297. The glycan contains up to 13 monomers of N-acetylglucosamine (GlcNAc, blue squares), fucose (red triangle), mannose (green circles), galactose (yellow circles), and sialic acid (pink diamonds). The linkages orientation of the monomers are indicated in red. PDB accession code 1IGY.

signaling modalities, as well as the neonatal Fc receptor (FcRn), which is involved in antibody recycling and therefore controls antibody half-life (Box 1). Collectively, interactions with different combinations of classical and non-classical Fc receptors result in distinct innate and adaptive immune cell responses, including ADCP, ADCC, complement-dependent cytotoxicity, and anti-inflammatory activity [12]. These effector functions have been implicated in protection against many viruses such as HIV, influenza, and Marburg [3,5,13–15], pointing to a critical need to understand how these functions are regulated immunologically *in vivo* in order to develop next-generation vaccine strategies able to harness the broad functional activity of the humoral immune response. While the intracellular synthetic pathways that lead to antibody glycosylation are well characterized, and a subset of glycan changes has been exploited to enhance monoclonal therapeutics, the immunologic mechanisms that control antibody glycosylation remain poorly understood. Here, we will review the mechanisms involved in antibody glycosylation, their functional impact, as well as the literature highlighting the natural variation of glycosylation in health and disease. Given the potential for glycosylation to benefit therapeutics, this review will discuss strategies to program and exploit glycosylation in the design of future therapeutics and vaccinations.

IgG Glycosylation As an Important Modulator of Antibody Functionality

IgG is composed of four polypeptides, two heavy chains each linked to a light chain that together form the antigen-binding domain (Fab). The constant/crystallizable region (Fc) is exclusively composed of the two heavy chains (Figure 1) [10,16]. While less variable than the Fab, the Fc domain is not constant, and changes both through the irreversible genomic selection of different protein backbones (isotype/subclass) and through post-translational glycosylation of Asn297 in the CH2 domain [10]. Humans possess four IgG subclasses and 36 possible antibody glycans, of which over 30 have been observed by mass spectrometry [17]. Thus, the potential combinatorial diversity of the constant domain allows up to 144

potential Fc regions for each specificity, and theoretically 144 different functional states (Figure 2, Key Figure) [17]. Whether all these combinations emerge naturally is incompletely known, but understanding the landscape of subclass and glycosylation changes in health and disease may provide critical new insights into novel Fc profiles that drive antibody function.

Box 1. Fc Receptors of the Immune System

Fc receptors, both activating and inhibitory, are expressed at variable levels and in different combinations on all cells of the innate immune system. The Fc receptors that bind IgG can be broken down into two broad classifications: Type I and Type II receptors or 'classical' and 'non-classical' receptors, respectively. The classical, Type I, receptors are immunoglobulin-family receptors, and include FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIb, of which all but FcγRIIb are activating receptors [11]. The Type II Fc receptors include the non-canonical receptors such as the C-lectin-type receptor DC-SIGN [11]. FcRn binds antibodies to transfer them across the placental barrier, as well as recycles antibodies in the serum [10,96]. The classical, Type I, Fc receptors bind close to the hinge, and are thus heavily influenced by the structure of the hinge, which is determined by antibody subclass and glycosylation (Figure I) [25,39]. Conversely, the non-classical, Type II, receptors generally bind IgG at a site between the two constant regions, CH2 and CH3 [25,39] (Figure I). As the shape of the CH2–CH3 interface is determined by the size of the glycan and the flexibility that the glycan confers to that region, these non-classical Fc receptors are also impacted by antibody flexibility. FcRn, uniquely, binds with a 2:1 stoichiometry, binding both CH2–CH3 interfaces of the antibody, and is insensitive to antibody glycosylation, with only a modest subclass preference for IgG1 [96].

Antibody binding to classical and non-classical Fc receptors is therefore impacted dramatically by the glycan attached to the Fc domain. Specific receptor–glycan interactions have been implicated in tuning the receptor–antibody affinity. For example, in the high-affinity FcγRI receptor, a loop of the Fc receptor extends into the Fc opening of the antibody and makes contact with glycans from both heavy chains of the antibody, primarily contacting the more proximal part of the glycan, an interaction absent in the low-affinity receptors [29]. Fucose can also directly interact with the glycans on FcγRIIIa, antagonizing binding [29]. This indicates that protein–protein, protein–glycan, and glycan–glycan interactions can be key to tuning antibody affinity.

The Fc receptors also differ in their expression patterns on immune cells. For example, FcγRI is expressed on monocytes/macrophages and dendritic cells, with inducible expression on neutrophils and mast cells [97]. FcγRIIa is more widely expressed, on all the aforementioned cells plus eosinophils and basophils. FcγRIIb is expressed on B cells, dendritic cells, and basophils, and inducible on monocytes/macrophages and neutrophils. FcγRIIc can be expressed on natural killer cells, monocytes/macrophages, and neutrophils [97]. FcγRIIIa is found on natural killer cells and monocyte/macrophages. FcγRIIIb is found on neutrophils and expressed at low levels on basophils [97]. Lastly, FcRn is found on monocytes/macrophages, dendritic cells, neutrophils, endothelial cells, and syncytiotrophoblasts [97]. This variation in expression allows differential responses to immune complexes from different cell subsets.

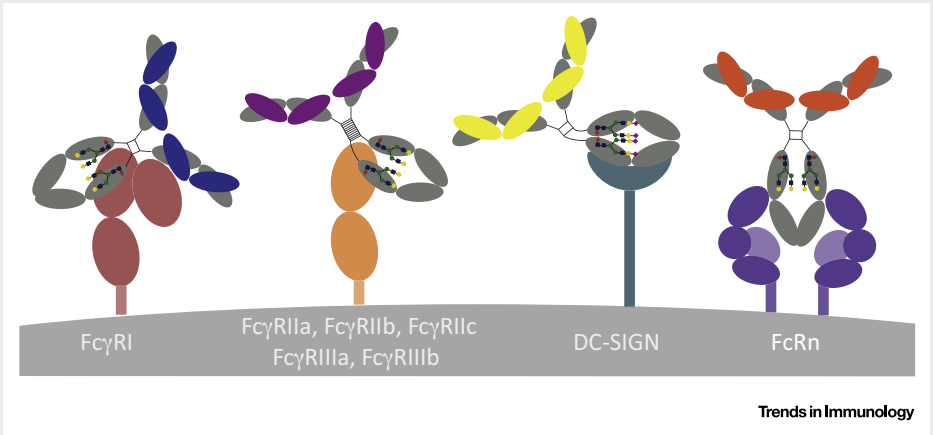

Figure I. Antibody Binding to Fc Receptors. Antibodies interact with Fc receptors in different ways. The classical, Type I, Fc receptors bind near the antibody hinge. Non-classical, Type II, Fc receptors including DC-SIGN and FcRn bind lower, below the CH2–CH3 interface. Abbreviation: FcRn, neonatal Fc receptor.

Key Figure

Antibody Combinatorial Diversity Drives Antibody Effector Function.

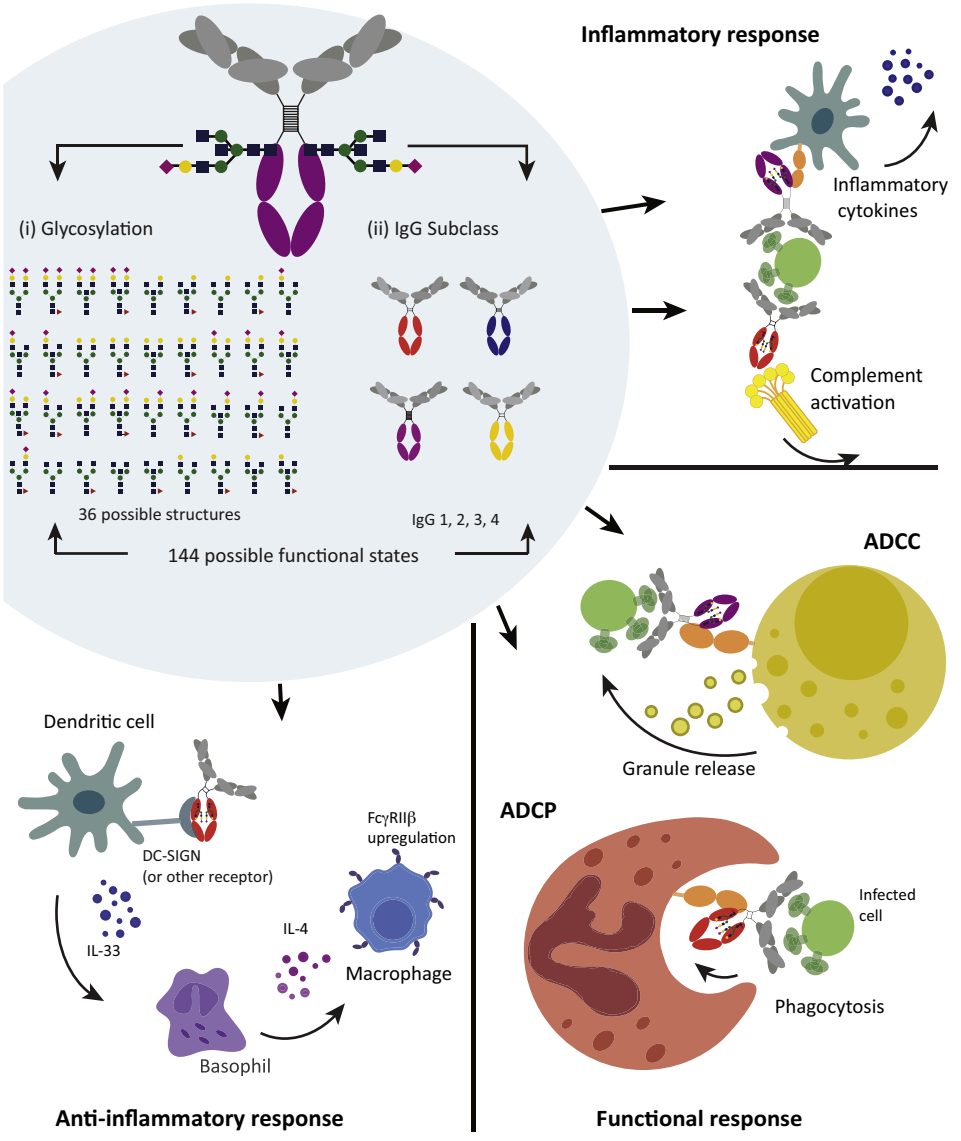

Figure 2. The IgG Fc is modified through two changes to the Fc domain of an antibody: (i) choice of glycosylation (36 options) and (ii) choice of subclass (four subclasses), creating 144 theoretical combinations and linked functional states. Depending on the antibody–glycan combination, many different functional responses may be elicited including the induction of an anti-inflammatory response; functional responses such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC); or inflammatory responses including complement activation and cytokine secretion. Abbreviation: IL, interleukin.

Table 1. Antibody Isotype Affinity for Fc Receptors [10,11,21,23,97]

Type	Serum abundance	FcγRI	FcγRIIa	FcγRIIb	FcγRIIc	FcγRIIIa	FcγRIIIb	FcRn
		Activating	Activating	Inhibitory	Activating	Activating	Activating	Recycling/placental transport
IgG1	60%	++++[a]	+++	++	++	+++	++	++++
IgG2	32%		++			+	+	++++
IgG3	4%	++++	+++	++	++	++++	+++	++/++++[b]
IgG4	4%	++++	++	++	++	++		++++

[a]+, K_A (M^{-1}): 10^4–10^5; ++, K_A (M^{-1}): 10^5–10^6; +++, K_A (M^{-1}): 10^6–10^7; ++++, K_A (M^{-1}): 10^7–10^8.
[b]IgG3 molecules of allotypes common in Caucasian communities have lower affinity for FcRn.

Antibody Subclass Selection and Functionality

The heavy-chain locus encodes multiple constant regions, including four IgG isotypes arranged in the following order: IgG3, IgG1, IgG2, and then IgG4. IgG1 is the main subclass, making up 60% of the serum IgG, with IgG2 at 32% and IgG3 and Ig4 both at 4% [10]. During isotype switching, selection of one subclass irreversibly loops out all upstream subclasses, so while IgG3 could switch forward to IgG1, IgG2, or IgG4 through further class-switch recombination, IgG1 cannot switch backward to IgG3 [18–20]. Interestingly, the subclass organization in humans matches their functional activity, based on Fc receptor affinity (Box 1): IgG3 has the highest affinity, then IgG1 with similarly high affinity before IgG2 and IgG4, both with much lower affinity (Table 1) [10,20,21]. However, it is worth noting that IgG3's short half-life limits its functionality in Caucasians, although longer-lived allotypes have been observed recently among non-Caucasian populations [22], related to hinge mutations that allow IgG3 to compete for binding to FcRn [23]. Moreover, the long hinge of IgG3 has been linked to enhanced complement activation, associated with efficient antibody–hexamer formation that activates complement [24]. Despite IgG3's superior affinity for Fc receptors, IgG1 is fifteen times more abundant in serum than IgG3, and therefore likely the dominant antibody effector molecule in circulation [10]. Conversely, IgG2 and IgG4 bind Fc receptors weakly and hence exhibit reduced functionality. Thus, pathogens, adjuvants, and T dependence can dictate subclass selection profiles, collectively influencing the evolution of the subclass response during infection, typically switching from the most functional subclasses to the least functional subclasses in humans. For example, when B cells respond to a T-dependent protein antigen, highly functional IgG3 and IgG1 antibodies are typically produced. Conversely, when B cells encounter a T-independent antigen, such as a carbohydrate, less functional IgG2 responses are typically elicited. Moreover, repeat exposure to the same antigen, as is observed for allergens, can drive the subclass response to our least functional antibody subclass, IgG4 [10]. This altered subclass selection program is likely an evolutionary mechanism to customize antibody effector function to the pathogenic insult or to homeostatically downregulate antibody effector function in the setting of chronic antigenic stimulation.

Changes in Antibody Glycosylation and Its Link to Antibody Effector Function

The IgG glycans are positioned between the heavy chains, and act as integral structural components of the Fc. The α1,6 arm makes non-covalent interactions with the Fc protein backbone and the α1,3 arm extends into the cavity between the heavy chains, interacting with the opposing glycan and influencing the antibody's structural rigidity and conformation [25,26]. While the Fc is always glycosylated, 20% of antibodies have additional glycosylation sites within the Fab, generated through somatic hypermutation, which have not been shown to influence Fc receptor binding [27]. By contrast, complete removal of the Fc glycan results in a near complete loss of classical, Type I, Fc receptor binding, with FcγRI retaining minimal binding [28,29].

While glycosylation is more varied on other proteins, the IgG glycan is always biantennary [30]. A high-mannose glycan is added to each heavy chain as it folds in the endoplasmic reticulum, which is first trimmed and then extended as the antibody transits through the Golgi (Box 2) [30]. The core glycan is composed of two sequential N-acetylglucosamine (GlcNAc) moieties attached at Asn297, to which a mannose is attached, followed by two additional mannose antennae, each capped with an additional GlcNAc (Figure 1) [30]. This forms the core antibody glycan structure, to which additional sugars can be added. The IgG glycan can be modified by the addition of a fucose to the primary GlcNAc, galactose to the antennary GlcNAcs, sialic acid to either additional galactose residue, and a bisecting GlcNAc (b-GlcNAc)

Box 2. Antibody Glycan Morphogenesis

Antibody glycans are assembled and attached to the antibody polypeptide chain co-translationally in the endoplasmic reticulum (ER) and modified in the Golgi apparatus. The glycan precursors are initially synthesized on the cytoplasmic side of the ER before being flipped to the inner leaflet and linked to a lipidlike molecule called a dolichol. As the antibody polypeptide is synthesized in the ER, the glycan precursor, $Glc_3Man_9GlcNAc_2$ (Figure I), is attached *en bloc* to Asn297 of the growing polypeptide chain by oligosaccharyltransferases. After the antibody passes through the protein-folding quality control, it is transported to the Golgi in a COPII-coated vesicle. As the nascent antibody transits through the Golgi, the glycan precursor is gradually trimmed by a highly ordered set of glycosidases to $Man_3GlcNAc_4$. This new precursor is then modified sequentially by a restricted set of four glycosyltransferases, which define the limited repertoire of antibody glycans. As the antibody moves through the Golgi, these enzymatically attach additional monosaccharaides to the precursor, leading to the potential development of up to 36 unique glycans that may be added to a secreted IgG (Figure I) [98].

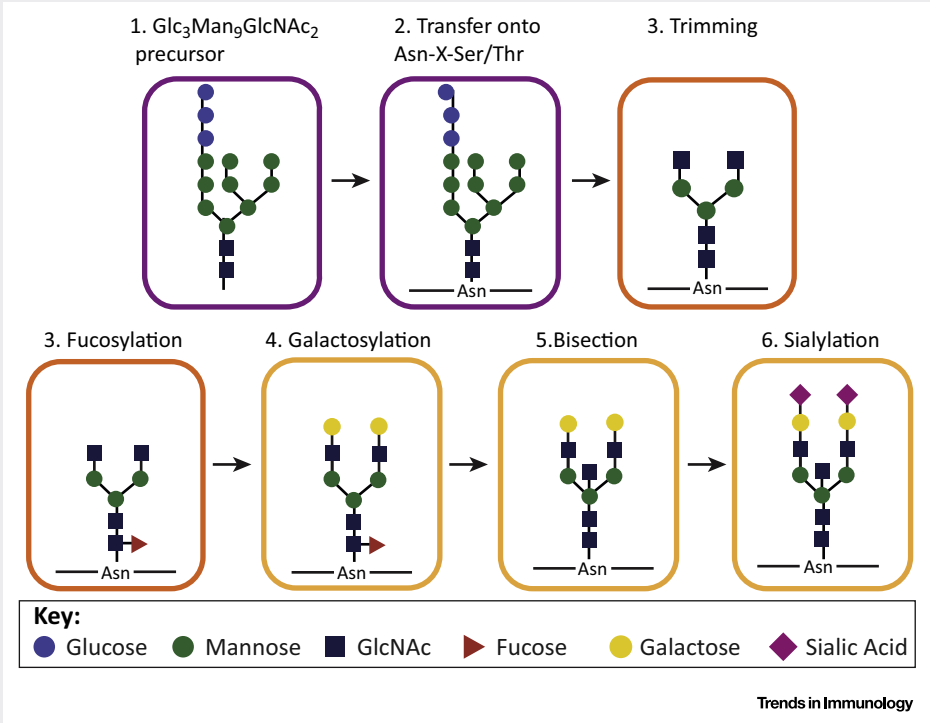

Figure I. Sequential Processing of the Antibody Glycan. The antibody glycan precursor, $Glc_3Man_9GlcNAc_2$, is transferred onto the antibody polypeptide in the endoplasmic reticulum (purple outline). Once the glycosylated polypeptide enters the Golgi, it interacts with a highly ordered sequence of glycosidases and glycosyltransferases that trim and extend the glycan into a classical antibody biantennary structure. The glycan is first trimmed into the Glc_4Man_3 precursor in a highly ordered and sequential manner within the *cis* and medial Golgi (orange outline). Then it is sequentially extended by four glycosyltransferases. FUT8 adds a fucose within the medial Golgi (orange outline), and then inside the *trans* Golgi (yellow outline) B4GALT1 adds one or two galactoses, MGAT3 adds the b-GlcNAc, and ST6GAL1 adds one or two sialic acids. Each glycan may or may not be modified by any specific glycosyltransferases, leading to 36 different versions of the glycan.

to the core mannose [30]. Ultimately, the variable addition of these sugars can generate up to 36 glycans (Figure 1) [30]. Moreover, the two heavy chains can be glycosylated asymmetrically, further increasing the combinatorial diversity of antibody glycosylation [31]. Recent research found that 70% of human IgG molecules were asymmetrically glycosylated, where pairing was preferentially homologous, but heterologous pairing was not random [31]. Specifically, this indicates that there are potential steric limitations, or preferential routing through the Golgi that allows certain glycoforms to pair [31]. The predominance of asymmetric Fc glycoforms points to a larger diversity of Fc structures that are not only dictated by a single glycan profile, but also by a larger repertoire of glycan combinations that may differentially drive antibody effector function.

The addition and removal of the variably added sugars (galactose, fucose, b-GlcNAc, or sialic acid) have been linked to altered antibody functionality. However, studies examining the functional impact of the removal of any single sugar likely are obscured by its impact on the addition of other sugars. For example, deficiencies in galactose will also impact sialylation, due to the need for galactose for the addition of terminal sialic acids [10]. Similarly, the addition of a b-GlcNAc will also lead to the downregulation of fucose levels due to steric hindrance of the enzyme [32]. Along these lines, an NMR study showed structural relaxation of the glycans with agalactosylation, which allowed binding of mannose-binding lectin directly to core GlcNAc sugars, and thus activation of the complement system [33]. Studies to support this observation have been limited. Yet, more recently, monoclonal antibody studies, using highly defined glycosylation profiles, pointed to a critical role of hypergalactosylation in driving ADCC via enhanced FcγRIIIa binding [34], suggesting that a single sugar may have diverging influences on different antibody functions. More concrete functional roles for other sugars have been documented.

Sialylation has been linked to anti-inflammatory effects. Intravenous immunoglobulin, used to treat rheumatoid arthritis (RA) and other inflammatory conditions, has anti-inflammatory effects driven by a population of antibodies bearing α2,6 sialylation [35,36]. Synthetic sialylation of Fcs has further replicated and confirmed the critical nature of antibody sialylation in anti-inflammatory activity [37]. Dissection of the mechanism(s) underlying this effect has been mixed. One model suggests that sialylation constrains the Fc region to a 'closed' conformation, reducing antibody affinity for classical Fc receptors, while enhancing affinity for the non-classical Fc receptor, DC-SIGN. DC-SIGN binding was shown to drive a cascade culminating in the production of interleukin-33 (IL-33) by dendritic cells, activating IL-4 expression by basophils, and ultimately upregulating the inhibitory FCγRIIb on macrophages, thereby nonspecifically dampening inflammation [38,39]. However, other structural analyses show that sialylation only marginally alters Fc domain structure [26], and minimally impacts DC-SIGN binding [40]. Other proposed models include the involvement of receptors such as Siglec/CD22 [41], the C-type lectin dendritic cell immunoreceptor [42], and the Fc receptor-like protein 5 [43] that may bind sialylated structures to drive anti-inflammatory responses. Though thought to be related to sialylation, the precise mechanism underlying intravenous immunoglobulin's anti-inflammatory effect remains unclear, and is under intense investigation.

Afucosylation directly boosts ADCC activity by enhancing FcγRIIIa binding through steric interactions with the FcγRIIIa glycan [44,45]. Specifically, fucosylation destabilizes the interaction between the antibody and FcγRIIIa's glycan, hindering binding [46,47]. Afucosylation allows carbohydrate–carbohydrate interactions that alleviate the steric clash and stabilize binding, increasing antibody affinity 100-fold [48]. Interestingly, b-GlcNAc also improves ADCC [6,49]. However, this mechanism appears dependent on b-GlcNAc's impact on fucosylation, rather than directly affecting Fc receptor binding. Specifically, bisection and fucosylation are largely mutually exclusive; a bisected antibody is unlikely to be fucosylated and inhibition of

fucosylation upregulates GntIII, the b-GlcNAc glycosyltransferase, which then inhibits downstream fucosylation [32,45,50]. Thus, bisection likely enhances antibody functionality via afucosylation, which improves antibody binding to FcγRIIIa.

Fc Functions of Antibodies

Antibodies may drive different effector functions, including ADCC, ADCP, CDC, and anti-inflammatory activity depending on the Fc receptors that they engage on in distinct subsets of innate immune cells. Yet, besides the high-affinity FcγRI (K_A: 10^8 M^{-1}), antibody affinity for other Fc receptors is low (K_A: 10^4–10^7 M^{-1}) [21]. To overcome this low affinity, activation of Fc receptors relies on coordinated binding of multiple antibodies to the target, forming high-valency immune complexes which bind and cluster Fc receptors on innate immune cells [51]. Since many Fc receptors may be engaged simultaneously, the balance of types of Fc receptors bound in the immune synapse determines the functional outcome of binding.

Activating receptors signal through an immunotyrosine activation motif (ITAM) either in the cytoplasmic tail (FcγRIIa/c, FcγRIIIb) or on the common gamma chain (all other Fc receptors) that clusters during activation, initiating signaling cascades that activate innate immune cells to induce antibody effector functions [52]. ITAM clustering activates the Src kinases that phosphorylate the ITAM, leading to signaling cascades that produce inositol triphosphate and diacylglycerol, causing calcium flux and phosphoinositide 3-kinase activation. These directly alter gene transcription through transcription factors such as nuclear factor of activated T cells, c-jun, c-fos, and nuclear factor-κB, initiating cytokine production and downstream activation [52,53]. Conversely, FcγRIIb has an immunotyrosine inhibition motif in its cytoplasmic tail, which, following Src-mediated phosphorylation, directly counteracts ITAM signaling, altering ITAM signaling partners through Erk pathway inhibition, and reducing phosphoinositide 3-kinase activation [52]. Upon activating Fc receptor and inhibitory FcγRIIb binding, activating and inhibitory signals are integrated within effector cells, ultimately aimed at directing innate immune effector function. However, the specific threshold or balance required to activate or to inhibit activity has yet to be defined and may be unique to individual innate immune effector cell types [53–55].

Antibodies also activate the complement cascade, similarly through low-affinity interactions [56]. Mechanistically, groups of six antibodies form hexamers that efficiently recruit C1q, the classical cascade initiator, or mannose-binding lectin, to the surface of infected cells or pathogens, activating downstream components and ultimately assembling the membrane attack complex [57,58]. Complement activation is inhibited on autologous cells, preventing immunopathology [59], but complement-induced antibody-opsonized cells may still be recognized and cleared by complement receptors on the surface of innate immune cells [59]. Moreover, because clearance can occur synergistically with Fc receptor cross-ligation, it can trigger either activation or inhibition of the immune response, depending on the type of receptors clustered [12].

Natural Glycovariation

Although glycosylation is highly ordered and conserved, disease-associated shifts documented in autoimmune and infectious diseases suggest that it is actively modulated during inflammatory responses [30,60,61]. However, the specific mechanisms that lead to variation in glycosylation are incompletely understood. Most likely, many cellular pathways shape antibody glycosylation, including glycosyltransferase/glycosidase expression, changes in monosaccharaide availability, Golgi pH, Golgi organization, kinetics of protein production, and availability of vesicular transport machinery. Broader factors, such as age, sex, and pregnancy also impact antibody glycosylation, arguing for hormonal control as well [17,62,63].

Autoimmune Disease Impacts Antibody Glycosylation

Many autoimmune conditions are associated with alterations in circulating antibody quantity and quality. In RA and lupus, patients' antibodies shift to a highly inflammatory glycosylation profile, marked by agalactosylation [64,65]. In these diseases, the new autoreactive, inflammatory antibodies are thought to contribute to disease pathology and inflammation through the destruction of autologous cells. In RA, agalactosylated antibodies arise prior to symptoms and are considered diagnostic for the condition [64], causing synovitis by accumulating within joints, activating macrophages via FcγRIIa, and promoting production of tumor necrosis factor-α (TNFα) and IL-6 [66]. Moreover, blockade of immune complex activation reduces TNFα and inflammation, strongly arguing that agalactosylation may be responsible for disease pathology [67]. The administration of aglycosylated antibodies, treated with EndoS, has been shown to alleviate and improve disease symptoms by preventing disease-associated immune complexes from binding to Fc receptors, thereby preventing immune activation and reducing pathology in diseases such as lupus [68].

Antibody Glycosylation Is Programmed in an Antigen-Specific Manner in Disease

Data from HIV infection have shown dramatic and persistent changes in antibody glycosylation [60,69]. Antibody glycosylation is rapidly and progressively altered over time, coming to resemble that seen in autoimmune diseases [60,69]. Given that hypergammaglobulinemia marks these chronic immune diseases [70], which have immune activation linked to an enrichment of agalactosylated antibodies [71], it is plausible that increased agalactosylation may simply be a marker of a nonspecific expansion of antibody-secreting cells, which may have an overwhelmingly agalactosylated profile, of which only a small fraction target the pathogen directly (Figure 3).

While agalactosylation levels normalize in RA patients when a disease flare resolves [61], agalactosylation does not resolve among HIV-infected patients with antiretroviral therapy and resolution of inflammation. These data suggest that B cells may be permanently altered, continuing to generate inflammatory glycans despite the complete blockade of viral replication [60]. Moreover, spontaneous controllers of HIV, who exhibit low to undetectable viral replication without antiretroviral therapy, maintain the highest levels of agalactosylated antibodies, suggesting that there could be a beneficial role for these inflammatory antibodies in the chronic spontaneous control of the virus. Changes in total antibody glycosylation are accompanied by changes in antigen-specific antibody glycosylation, though these appear to be independent

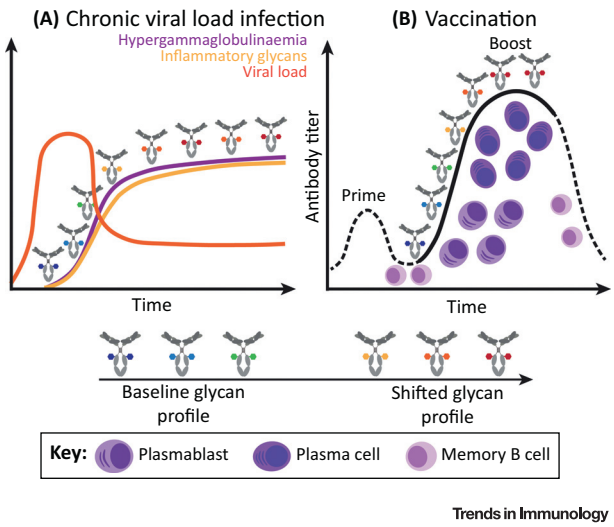

Figure 3. Glycan Changes in Disease and Following Vaccination. (A) Over the course of HIV infection, antibodies shift toward a more inflammatory profile, in parallel with the evolution of hypergammaglobulinemia [60,70]. (B) Following vaccination, influenza-specific antibodies shift from pre-vaccine antibody glycan profiles toward a distinct glycan profile that is thought to regulate the overall humoral immune response to vaccination [72,88]. Whether these overall shifts in these models are related to the generation of plasmablasts, or the conversion of memory B cells into plasmablasts, is uncertain at this time, but appears to be regulated in an antigen-specific manner. The blue to red color scale of the Fc glycan depicts the overall change from basal circulating nonspecific glycans (dark blue) to highly modified glycans (red).

[69]. This suggests that glycosylation of pathogen-specific antibodies occurs independently of overall inflammatory changes on total circulating antibodies [60,69]. Furthermore, following vaccination with an experimental HIV vaccine, HIV-specific antibodies exhibited a distinct glycan profile, compared to total antibody [69]. Moreover, these vaccine-induced antigen-specific antibody glycan profiles are nearly identical in vaccinees across the globe, despite significant differences in the total antibody glycosylation profiles globally [69,72]. Collectively, these data indicate that while there are a variety of factors that impact baseline antibody glycosylation, the specificity of the changes after vaccination argues for a common and precise regulation of glycosylation at the time of immune priming.

Genetic and Environmental Contributions to Natural Variation in Glycosylation

Large-scale population screening and genome-wide association studies around the world have demonstrated that genetics and diet significantly influence antibody glycosylation [17,73]. Collectively, glycosylation is around 50% heritable. Among the individual sugars, sialylation is particularly heritable, indicating that some sugars are under stricter genetic regulation. Antibody glycosylation is influenced by a multitude of genes consisting of almost 5% of the human genome that fall into many different pathways including cell metabolism, endoplasmic reticulum activity, Golgi apparatus dynamics, and secretion, highlighting the diverse pathways and complex regulation that govern glycosylation [74]. Genome-wide association studies have also identified several specific genes involved in antibody glycosylation. HNF1A, a polymorphic transcription factor linked to diabetes and glucose metabolism, was identified as a master regulator of fucosylation, promoting salvage and synthesis of fucose precursors and suppressing the fucosyltransferase FUT8. SLC9A9, a proton pump, is connected to sialylation, potentially altering Golgi pH to relocated sialyltransferases [75,76]. Other identified loci overlap with hematological cancer loci, including *IL6ST*, a cytokine signal transducer, associated with agalactosylation and digalactosylation, indicating the importance of cytokines in the control of glycosylation [77]. The *SMARCB1–DERL3* loci, coding for two proteins related to tumor inhibition and the misfolded protein response, respectively, has also been associated with variation in b-GlcNAc and G1F. Finally, the DNA-binding protein, Ikaros, was shown to influence fucosylation [77]. Critically, while these polymorphisms mark changes in the overall levels of circulating IgG glycosylation, they may not directly affect antibody glycosylation or glycosylation machinery, further emphasizing the need to define the mechanisms of control for glycosylation.

Age has also been linked to changes in antibody glycosylation, where antibody agalactosylation doubles (at the expense of digalactosylation) and b-GlcNAc increases over a lifetime [17,78]. Sex-associated patterns are also significant; women show more dynamic age-related glycosylation changes, and begin to lose digalactosylation at menopause, while men exhibit a steady decline throughout their lifetime [79]. Pregnancy-associated variation has also been noted, including a significant reduction in agalactosylated and fucosylated antibody species, further suggesting a role for hormonal control of glycosylation [17,79]. However, many additional factors have been implicated in shaping glycosylation including body mass index, smoking, and biochemistry, collectively accounting for an additional 5% of the variation observed in glycosylation profile changes across populations, highlighting the breadth of factors that impact the antibody glycome [80].

Opportunities to Utilize Antibody Glycosylation to Design Better Vaccines

The discovery that a correlate of protection for the first moderately protective HIV vaccine, RV144, was HIV-specific antibodies able to simultaneously direct multiple effector functions led to the discovery that the vaccine elicited elevated levels of HIV-specific IgG3 antibodies, pointing to the potential importance of Fc effector-mediating antibodies as a novel mechanism of protection against HIV [81,82]. Likewise, vaccine studies in the animal model of HIV, non-human primates, support a role for functional antibodies in protection from infection

[13]. However, antibody subclass alone does not predict protection against HIV, or its non-human primate analog SIV, as depletion of IgG3 from RV144 plasma samples incompletely reduced antibody functionality. This suggests that additional antibodies, including subpopulations of IgG1, are important contributors to antibody function [81]. Moreover, Fc effector function has been linked to protection in additional infections including influenza, Marburg virus, *Bacillus anthracis*, and *Mycobacterium tuberculosis* [5,83–85]. Thus, with the growing appreciation for the importance of antibody glycosylation in driving antibody functionality, and the importance of antibody function in the control of multiple pathogens, defining the immunological mechanism(s) that actively tune this post-translational modification may provide novel insights for the design of next-generation immunogens.

HIV As a Model for Glycosylation During Natural Infection

Because of the growing interest in antibody effector function in the control and eradication of HIV, in-depth characterization has been applied to define the mechanisms by which some individuals are able to generate and sustain high levels of functional antibodies and why these antibodies have broad antiviral effects [4]. Significant differences in antibody glycosylation exist among distinct HIV patient phenotypes [60,86], with an accumulation of agalactosylated antibodies that is most dramatic in spontaneous controllers of HIV. Interestingly, these agalactosylated profiles are also enriched on HIV-specific antibodies in 'controllers', who also exhibit lower fucosylation and enhanced viral suppression [60]. Remarkably, other glycosylation changes have been observed in HIV infection, where noncontrollers, treated to reduce viral replication, generate HIV-specific antibodies with enhanced functional activity through the production of b-GlcNAc antibodies [60]. Thus, emerging data suggest that control of HIV may be achieved via the induction of functional antibodies against the virus both through the expansion of highly functional IgG3 antibodies and through the generation of selectively glycosylated IgG1 antibodies, which dwarf IgG3 antibodies in abundance and potentially offer similarly enhanced functionality [4,87]. These findings argue that the mechanism(s) that control the preferential induction of protective humoral immunity, driven via unique subclass and glycan selection, may be understood through the analysis of the humoral immune response among spontaneous controllers.

Antigen-Specific Glycosylation

While emerging data suggest that antibody glycosylation may be controlled in an antigen-specific manner [69,72,88], data supporting the possibility of directly controlling this activity through vaccination are scarce. Preliminary studies of vaccine-induced antibodies have begun to highlight how antibody glycosylation may be regulated [69,72]. Specifically, pioneering work showed that the glycosylation of influenza-specific and tetanus-specific antibodies, but not total antibodies, changed upon vaccination, indicating that only responding B cells modulated glycosylation patterns [72]. Vaccination increased galactosylation and sialylation without changes in other sugars, highlighting differential regulation of select sugars [72]. However, these changes were transient, likely related to a rapid response to B cell activation, returning to baseline after 9 months (Figure 3) [72]. Likewise, recent studies following influenza vaccination showed that a rapid, transient increase in antibody sialylation correlated with higher affinity antibodies. Mechanistically, sialylated antibodies engaged CD23 and induced the inhibitory receptor FcγRIIb, which raised the threshold for receptor B cell activation [88]. This study highlighted an intriguing role for antibody glycosylation in driving higher quality immune responses. As both of these studies focused on recall vaccine responses, they highlight glycosylation changes following boosting, which may be distinct from those occurring during initial responses.

By contrast, studies using novel antigens, including HIV envelope proteins, highlight a unique opportunity to directly program and skew antibody glycosylation. Specifically, while

glycosylation profiles on total circulating IgG antibodies were different across three vaccination sites (USA, East Africa, and South Africa), an experimental HIV vaccine drove a single, highly homogenous antibody glycosylation profile on antigen-specific antibodies [69]. These data suggest that the vaccine-induced priming signal likely overcame baseline glycosylation differences, resulting in the induction of a single glycosylation profile. How this occurs is incompletely understood, but the data suggest that specific inflammatory signatures during priming may be integrated and utilized to direct antibody glycosylation. In the same study, nonspecific antibodies (antihemagglutinin) did not show an altered glycosylation profile upon vaccination, highlighting the specific programming of antigen-specific B cells [69]. These data collectively support a model whereby B cell priming may also select and fine-tune antibody glycosylation specific to the vaccine/pathogen signals delivered at the time of priming. Whether these glycan profiles are permanently locked into memory is unknown, but recall profiling suggests that boosting transiently and specifically alters antigen-specific glycosylation, arguing that glycosylation may shift in early plasmablasts to tune immunity; however, it rapidly returns to the originally programmed profile (Figure 3) [72]. Thus, these data highlight that immunological control exists in antibody glycosylation, likely regulated within antigen-specific B cells through antigen exposure at the time of priming and recall. Unlocking the rules by which glycosylation is controlled immunologically may reveal novel opportunities for the development of vaccines that harness Fc effector functions by selectively promoting certain glycosylation patterns.

Outstanding Questions

How do individual glycoforms interact with Fc receptors, and change the binding profile of the antibody?

What signals program the selection of a specific antibody B cell glycosylation state?

To what extent can antibody glycosylation be programmed by immunogen selection?

Once programmed, how is glycosylation committed to memory, and how stable is a given profile?

Programming Antibody Glycosylation

As described earlier, distinct glycosylation profiles on antigen-specific antibodies suggest that glycosylation may be programmed during B cell priming, potentially to customize antibody effector function in a pathogen-/antigen-specific manner [89]. Along these lines, *in vitro* B cell stimulation was shown to selectively alter antibody glycosylation. Specifically, IL-21 and CpG enhanced galactosylation and sialylation, while all-*trans* retinoic acid had the opposite effect [89]. While these factors are linked to B cell survival and proliferation, not all growth factors, including IL-4 or TNFα, influenced glycosylation [89], arguing that glycosylation is regulated independently of maturation [90]. Moreover, because antibody glycosylation is controlled independent of cell-wide glycosylation, the data suggest that it is controlled temporally or via Golgi compartmentalization [89,91]. This regulation of glycosylation could be achieved in many ways. For example, changes in antibody glycosylation following influenza vaccination were linked to changing glycosyltransferase levels that were potentially connected to cellular activation and/or inflammation [72,88]. Other regulatory pathways may elicit unique antigen-specific antibody glycosylation due to epigenetic, transcriptional, and post-transcriptional programming at the antigen-specific B cell level following immunologic priming. Programming of glycosylation could occur through modifications to enzymatic, carrier, or Golgi/vesicular apparatus machinery; changes in pH-dependent inactivation and recycling of glycosyltransferases through endosomes; regulation of glycosyltransferase expression by microRNAs; changes in cellular metabolism and availability of sugar nucleotides; aspartyl protease cleavage of membrane-bound glycosyltransferases into soluble forms; and secretion of glycosidases into the serum [76,92–95]. Thus, the process of programming glycosylation may be broad and varied, warranting deeper investigation to define the mechanisms by which glycosylation is naturally fine-tuned to regulate antibody functional activity.

Concluding Remarks and Future Perspective

Emerging evidence suggests that antibody glycosylation is actively controlled immunologically, representing a novel approach to direct immune regulation and antibody functional activity. However, the pathways that control this activity are incompletely understood. Future studies aimed at dissecting the immunological signaling during B cell priming will be key to deconvoluting the mechanisms underlying antigen-specific antibody glycosylation (see Outstanding Questions). Moreover, determining the cellular players that regulate B cell functional

priming will be critically important to identify the cellular circuits involved in regulating antibody effector activity. Collectively, defining the specific signals that program antibody effector function will provide novel insights for the design of next-generation vaccines able to induce designer glycan profiles with linked effector functions. This will allow the generation of targeted therapies for a broad array of infectious, oncological, and autoimmune diseases for which traditional vaccinology approaches or monoclonal therapeutic design efforts have failed.

References

1. Plotkin, S.A. (2010) Correlates of protection induced by vaccination. *Clin. Vaccine Immunol.* 17, 1055–1065
2. Excler, J.L. *et al.* (2014) Nonneutralizing functional antibodies: a new "old" paradigm for HIV vaccines. *Clin. Vaccine Immunol.* 21, 1023–1036
3. Teo, A. *et al.* (2016) Functional antibodies and protection against blood-stage Malaria. *Trends Parasitol.* 32, 887–898
4. Ackerman, M.E. *et al.* (2016) Polyfunctional HIV-specific antibody responses are associated with spontaneous HIV control. *PLoS Pathog.* 12, e1005315
5. Kajihara, M. *et al.* (2012) Inhibition of Marburg virus budding by nonneutralizing antibodies to the envelope glycoprotein. *J. Virol.* 86, 13467–13474
6. Goede, V. *et al.* (2014) Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N. Engl. J. Med.* 370, 1101–1110
7. Junttila, T.T. *et al.* (2010) Superior *in vivo* efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer. *Cancer Res.* 70, 4481–4489
8. Sondermann, P. and Szymkowski, D.E. (2016) Harnessing Fc receptor biology in the design of therapeutic antibodies. *Curr. Opin. Immunol.* 40, 78–87
9. Scott, A.M. *et al.* (2012) Antibody therapy of cancer. *Nat. Rev. Cancer* 12, 278–287
10. Vidarsson, G. *et al.* (2014) IgG subclasses and allotypes: from structure to effector functions. *Front Immunol.* 5, 520
11. Pincetic, A. *et al.* (2014) Type I and type II Fc receptors regulate innate and adaptive immunity. *Nat. Immunol.* 15, 707–716
12. Takai, T. (2002) Roles of Fc receptors in autoimmunity. *Nat. Rev. Immunol.* 2, 580–592
13. Hessell, A.J. *et al.* (2007) Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* 449, 101–104
14. Ahmad, R. *et al.* (2001) Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti-HIV-1 antibodies and prognostic predictors of HIV infection. *J. Clin. Immunol.* 21, 227–233
15. Co, M.D. *et al.* (2014) Relationship of preexisting influenza hemagglutination inhibition, complement-dependent lytic, and antibody-dependent cellular cytotoxicity antibodies to the development of clinical illness in a prospective study of A (H1N1)pdm09 influenza in children. *Viral Immunol.* 27, 375–382
16. Schroeder, H.W., Jr and Cavacini, L. (2010) Structure and function of immunoglobulins. *J. Allergy Clin. Immunol.* 125, S41–S52
17. Pucic, M. *et al.* (2011) High throughput isolation and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations. *Mol. Cell Proteomics* 10, M111.010090
18. Matthews, A.J. *et al.* (2014) Regulation of immunoglobulin class-switch recombination: choreography of noncoding transcription, targeted DNA deamination, and long-range DNA repair. *Adv. Immunol.* 122, 1–57
19. Jung, S. *et al.* (1993) Shutdown of class switch recombination by deletion of a switch region control element. *Science* 259, 984–987
20. Jung, D. *et al.* (2006) Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu. Rev. Immunol.* 24, 541–570
21. Bruhns, P. *et al.* (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* 113, 3716–3725
22. Einarsdottir, H. *et al.* (2014) H435-containing immunoglobulin G3 allotypes are transported efficiently across the human placenta: implications for alloantibody-mediated diseases of the newborn. *Transfusion* 54, 665–671
23. Stapleton, N.M. *et al.* (2011) Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. *Nat. Commun.* 2, 599
24. Rosner, T. *et al.* (2013) An IgG3 switch variant of rituximab mediates enhanced complement-dependent cytotoxicity against tumour cells with low CD20 expression levels. *Br. J. Haematol.* 161, 282–286
25. Krapp, S. *et al.* (2003) Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J. Mol. Biol.* 325, 979–989
26. Ahmed, A.A. *et al.* (2014) Structural characterization of anti-inflammatory immunoglobulin G Fc proteins. *J. Mol. Biol.* 426, 3166–3179
27. van de Bovenkamp, F.S. (2016) The emerging importance of IgG Fab glycosylation in immunity. *J Immunol.* 196, 1435–1441
28. Subedi, G.P. and Barb, A.W. (2015) The structural role of antibody N-glycosylation in receptor interactions. *Structure* 23, 1573–1583
29. Lu, J. *et al.* (2015) Structure of FcgammaRI in complex with Fc reveals the importance of glycan recognition for high-affinity IgG binding. *Proc. Natl. Acad. Sci. U. S. A.* 112, 833–838
30. Stanley, P. *et al.* (2009) N-glycans. In *Essentials of Glycobiology* (2nd edn) (Varki, A., ed.), Cold Spring Harbor Laboratory Press
31. Masuda, K. *et al.* (2000) Pairing of oligosaccharides in the Fc region of immunoglobulin G. *FEBS Lett.* 473, 349–357
32. Nishima, W. *et al.* (2012) Effect of bisecting GlcNAc and core fucosylation on conformational properties of biantennary complex-type N-glycans in solution. *J. Phys. Chem. B* 116, 8504–8512
33. Malhotra, R. *et al.* (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat. Med.* 1, 237–243
34. Thomann, M. *et al.* (2016) Fc-galactosylation modulates antibody-dependent cellular cytotoxicity of therapeutic antibodies. *Mol. Immunol.* 73, 69–75
35. Anthony, R.M. *et al.* (2008) Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science* 320, 373–376
36. Kaneko, Y. *et al.* (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 313, 670–673
37. Washburn, N. *et al.* (2015) Controlled tetra-Fc sialylation of IVIg results in a drug candidate with consistent enhanced anti-inflammatory activity. *Proc. Natl. Acad. Sci. U. S. A.* 112, E1297–E1306
38. Anthony, R.M. *et al.* (2011) Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature* 475, 110–113
39. Sondermann, P. *et al.* (2013) General mechanism for modulating immunoglobulin effector function. *Proc. Natl. Acad. Sci. U. S. A.* 110, 9868–9872
40. Yu, X. *et al.* (2013) Dissecting the molecular mechanism of IVIg therapy: the interaction between serum IgG and DC-SIGN is

independent of antibody glycoform or Fc domain. *J. Mol. Biol.* 425, 1253–1258

41. Seite, J.F. *et al.* (2010) IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. *Blood* 116, 1698–1704
42. Massoud, A.H. *et al.* (2014) Dendritic cell immunoreceptor: a novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells. *J. Allergy Clin. Immunol.* 133, 853–863 e5
43. Haga, C.L. *et al.* (2007) Fc receptor-like 5 inhibits B cell activation via SHP-1 tyrosine phosphatase recruitment. *Proc. Natl. Acad. Sci. U. S. A.* 104, 9770–9775
44. Shields, R.L. *et al.* (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. *J. Biol. Chem.* 277, 26733–26740
45. Shinkawa, T. *et al.* (2003) The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *J. Biol. Chem.* 278, 3466–3473
46. Sondermann, P. *et al.* (2000) The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc gammaRIII complex. *Nature* 406, 267–273
47. Ferrara, C. *et al.* (2006) The carbohydrate at FcgammaRIIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. *J. Biol. Chem.* 281, 5032–5036
48. Ferrara, C. *et al.* (2011) Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. *Proc. Natl. Acad. Sci. U. S. A.* 108, 12669–12674
49. Davies, J. *et al.* (2001) Expression of GnTIII in a recombinant anti-CD20 CHO production cell line: expression of antibodies with altered glycoforms leads to an increase in ADCC through higher affinity for FC gamma RIII. *Biotechnol. Bioeng.* 74, 288–294
50. Kurimoto, A. *et al.* (2014) The absence of core fucose up-regulates GnT-III and Wnt target genes: a possible mechanism for an adaptive response in terms of glycan function. *J. Biol. Chem.* 289, 11704–11714
51. Goodridge, H.S. *et al.* (2012) Mechanisms of Fc receptor and dectin-1 activation for phagocytosis. *Traffic* 13, 1062–1071
52. Getahun, A. and Cambier, J.C. (2015) Of ITIMs, ITAMs, and ITAMis: revisiting immunoglobulin Fc receptor signaling. *Immunol. Rev.* 268, 66–73
53. van Egmond, M. *et al.* (2015) Cross-talk between pathogen recognizing Toll-like receptors and immunoglobulin Fc receptors in immunity. *Immunol. Rev.* 268, 311–327
54. Boruchov, A.M. *et al.* (2005) Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* 115, 2914–2923
55. Billadeau, D.D. and Leibson, P.J. (2002) ITAMs versus ITIMs: striking a balance during cell regulation. *J. Clin. Invest.* 109, 161–168
56. Karsten, C.M. and Kohl, J. (2012) The immunoglobulin, IgG Fc receptor and complement triangle in autoimmune diseases. *Immunobiology* 217, 1067–1079
57. Noris, M. and Remuzzi, G. (2013) Overview of complement activation and regulation. *Semin. Nephrol.* 33, 479–492
58. Diebolder, C.A. *et al.* (2014) Complement is activated by IgG hexamers assembled at the cell surface. *Science* 343, 1260–1263
59. Zipfel, P.F. and Skerka, C. (2009) Complement regulators and inhibitory proteins. *Nat. Rev. Immunol.* 9, 729–740
60. Ackerman, M.E. *et al.* (2013) Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. *J. Clin. Invest* 123, 2183–2192
61. Pasek, M. *et al.* (2006) Galactosylation of IgG from rheumatoid arthritis (RA) patients – changes during therapy. *Glycoconj. J.* 23, 463–471
62. Kapur, R. *et al.* (2014) A prominent lack of IgG1-Fc fucosylation of platelet alloantibodies in pregnancy. *Blood* 123, 471–480
63. Menni, C. *et al.* (2013) Glycosylation of immunoglobulin G: role of genetic and epigenetic influences. *PLoS One* 8, e82558
64. Parekh, R.B. *et al.* (1985) Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature* 316, 452–457
65. Vuckovic, F. *et al.* (2015) Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome. *Arthritis Rheumatol.* 67, 2978–2989
66. Clavel, C. *et al.* (2008) Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. *Arthritis Rheum.* 58, 678–688
67. Ben Mkaddem, S. *et al.* (2014) Shifting FcgammaRIIA-ITAM from activation to inhibitory configuration ameliorates arthritis. *J. Clin. Invest.* 124, 3945–3959
68. Lood, C. *et al.* (2012) IgG glycan hydrolysis by endoglycosidase S diminishes the proinflammatory properties of immune complexes from patients with systemic lupus erythematosus: a possible new treatment? *Arthritis Rheum.* 64, 2698–2706
69. Mahan, A.E. *et al.* (2016) Antigen-specific antibody glycosylation is regulated via vaccination. *PLoS Pathog.* 12, e1005456
70. De Milito, A. *et al.* (2004) Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. *Blood* 103, 2180–2186
71. de Jong, S.E. *et al.* (2016) IgG1 Fc N-glycan galactosylation as a biomarker for immune activation. *Sci. Rep.* 6, 28207
72. Selman, M.H. *et al.* (2012) Changes in antigen-specific IgG1 Fc N-glycosylation upon influenza and tetanus vaccination. *Mol. Cell. Proteomics* 11, M111.014563
73. Knezevic, A. *et al.* (2009) Variability, heritability and environmental determinants of human plasma N-glycome. *J. Proteome Res.* 8, 694–701
74. Zoldos, V. *et al.* (2013) Genomics and epigenomics of the human glycome. *Glycoconj. J.* 30, 41–50
75. Huffman, J.E. *et al.* (2011) Polymorphisms in B3GAT1, SLC9A9 and MGAT5 are associated with variation within the human plasma N-glycome of 3533 European adults. *Hum. Mol. Genet.* 20, 5000–5011
76. Rivinoja, A. *et al.* (2009) Elevated Golgi pH impairs terminal N-glycosylation by inducing mislocalization of Golgi glycosyltransferases. *J. Cell. Physiol.* 220, 144–154
77. Lauc, G. *et al.* (2013) Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. *PLoS Genet.* 9, e1003225
78. Kristic, J. *et al.* (2014) Glycans are a novel biomarker of chronological and biological ages. *J. Gerontol. A Biol. Sci. Med. Sci.* 69, 779–789
79. Chen, G. *et al.* (2012) Human IgG Fc-glycosylation profiling reveals associations with age, sex, female sex hormones and thyroid cancer. *J. Proteomics* 75, 2824–2834
80. Knezevic, A. *et al.* (2010) Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans. *Glycobiology* 20, 959–969
81. Chung, A.W. *et al.* (2014) Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci. Transl. Med.* 6, 228ra38
82. Yates, N.L. *et al.* (2014) Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci. Transl. Med.* 6, 228ra39
83. Carragher, D.M. *et al.* (2008) A novel role for non-neutralizing antibodies against nucleoprotein in facilitating resistance to influenza virus. *J. Immunol.* 181, 4168–4176
84. Abboud, N. *et al.* (2010) A requirement for FcgammaR in antibody-mediated bacterial toxin neutralization. *J. Exp. Med.* 207, 2395–2405
85. Lu, L.L. *et al.* (2016) A functional role for antibodies in tuberculosis. *Cell* 167, 433–443 e14
86. Moore, J.S. *et al.* (2005) Increased levels of galactose-deficient IgG in sera of HIV-1-infected individuals. *AIDS* 19, 381–389

87. Wren, L.H. *et al.* (2013) Specific antibody-dependent cellular cytotoxicity responses associated with slow progression of HIV infection. *Immunology* 138, 116–123

88. Wang, T.T. *et al.* (2015) Anti-HA glycoforms drive B cell affinity selection and determine influenza vaccine efficacy. *Cell* 162, 160–169

89. Wang, J. *et al.* (2011) Fc-glycosylation of IgG1 is modulated by B-cell stimuli. *Mol. Cell. Proteomics* 10, M110.004655

90. Omtvedt, L.A. *et al.* (2006) Glycan analysis of monoclonal antibodies secreted in deposition disorders indicates that subsets of plasma cells differentially process IgG glycans. *Arthritis Rheum.* 54, 3433–3440

91. Moremen, K.W. *et al.* (2012) Vertebrate protein glycosylation: diversity, synthesis and function. *Nat. Rev. Mol. Cell Biol.* 13, 448–462

92. Voss, M. *et al.* (2014) Shedding of glycan-modifying enzymes by signal peptide peptidase-like 3 (SPPL3) regulates cellular N-glycosylation. *EMBO J.* 33, 2890–2905

93. Bernardi, C. *et al.* (2013) Effects of microRNAs on fucosyltransferase 8 (FUT8) expression in hepatocarcinoma cells. *PLoS One* 8, e76540

94. Novokmet, M. *et al.* (2014) Changes in IgG and total plasma protein glycomes in acute systemic inflammation. *Sci. Rep.* 4, 4347

95. Jones, M.B. *et al.* (2012) Anti-inflammatory IgG production requires functional P1 promoter in beta-galactoside alpha2,6-sialyltransferase 1 (ST6Gal-1) gene. *J. Biol. Chem.* 287, 15365–15370

96. Roopenian, D.C. and Akilesh, S. (2007) FcRn: the neonatal Fc receptor comes of age. *Nat. Rev. Immunol.* 7, 715–725

97. Bruhns, P. (2012) Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 119, 5640–5649

98. Arnold, J.N. *et al.* (2007) The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* 25, 21–50