REVIEW

Fc gamma receptors: glycobiology and therapeutic prospects

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Abstract: Therapeutic antibodies hold great promise for the treatment of cancer and autoimmune diseases, and developments in antibody–drug conjugates and bispecific antibodies continue to enhance treatment options for patients. Immunoglobulin (Ig) G antibodies are proteins with complex modifications, which have a significant impact on their function. The most important of these modifications is glycosylation, the addition of conserved glycans to the antibody Fc region, which is critical for its interaction with the immune system and induction of effector activities such as antibody-dependent cell cytotoxicity, complement activation and phagocytosis. Communication of IgG antibodies with the immune system is controlled and mediated by Fc gamma receptors (FcγRs), membrane-bound proteins, which relay the information sensed and gathered by antibodies to the immune system. These receptors are also glycoproteins and provide a link between the innate and adaptive immune systems. Recent information suggests that this receptor glycan modification is also important for the interaction with antibodies and downstream immune response. In this study, the current knowledge on FcγR glycosylation is discussed, and some insight into its role and influence on the interaction properties with IgG, particularly in the context of biotherapeutics, is provided. For the purpose of this study, other Fc receptors such as FcαR, FcεR or FcRn are not discussed extensively, as IgG-based antibodies are currently the only therapeutic antibody-based products on the market. In addition, FcγRs as therapeutics and therapeutic targets are discussed, and insight into and comment on the therapeutic aspects of receptor glycosylation are provided.

Keywords: glycosylation, IgG, Fc gamma receptor, therapeutic monoclonal antibody

Therapeutic antibodies and glycosylation

Antibodies or immunoglobulins (Igs) are important components of the humoral immune system, which act as surveyors, sensing pathogens and transformed cells, communicating this information to the innate and adaptive immune systems. IgG antibodies provide the first line of defense against invading microorganisms, and due to their ability to detect tumor-associated antigens and neutralize inflammatory mediators such as tumor necrosis factor (TNF)-α this class of antibodies has been used with great success in treatments for cancer and autoimmunity conditions. Therapeutically, all the current monoclonal antibodies (Mabs) and Mab fusion proteins used in autoimmune diseases, inflammatory conditions and oncology use the IgG backbone. This is the most studied and best characterized of the Igs and is divided into four distinct subclasses (IgG1, IgG2, IgG3, IgG4), each with differences in sequence and structure, binding properties to cellular Fc gamma receptors (FcγRs) and effector functions (Figure 1).[1,2] Mab therapy was born in the 1970s with the major discoveries of the IgG structure by Edelman et al[3]
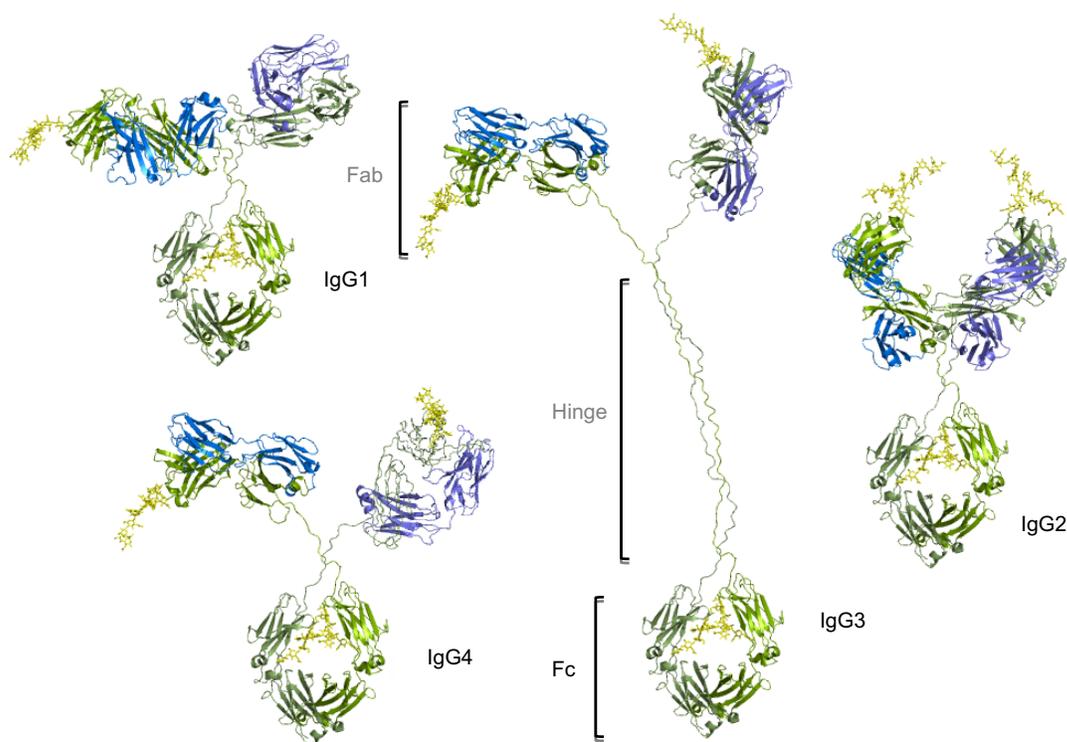
Figure 1 The IgG subtypes.
Notes: Four subtypes of IgG exist in humans: IgG1, IgG2, IgG3, IgG4, each with differences in sequence, structure, glycosylation and communication with FcγRs. The four subtypes are named based on their respective abundance in serum with IgG1 being the most abundant. IgG antibodies consist of two Fab regions that can bind both an antigen molecule and the Fc region which interact with the FcγR, joined by a highly flexible hinge region. IgG has a longer hinge region than the other IgG subtypes. Each IgG subtype has conserved Asn 297 amino acids in the Fc region with N-glycans attached (shown in yellow). Typically, the Fc glycans are bi-antennary galactosylated structures with varying amounts of core fucosylation and sialylation. Glycosylation is also found in the Fab regions with higher proportions of galactosylated and sialylated glycans. Heavy chains are shown in green, and light chains are shown in blue and purple.
Abbreviations: Fab, fragment antigen binding; FcγR, Fc gamma receptor; IgG, immunoglobulin G.

and Porter[4] and the development of hybridoma technology by Kohler and Milstein.[5] Initially, Mab therapeutics were murine in nature, leading to significant problems such as inadequate serum retention, induction of IgE-specific allergic reactions and anaphylaxis due to the presence of murine-derived gal α(1,3)-gal and N-glycolylneuraminic acid glycan epitopes and failure to induce effector responses through impaired interaction with human FcγRs.[6] Developments in recombinant antibody technology and the production of chimeric, humanized and fully human antibodies have addressed many of these issues, most importantly the humanization of glycosylation to ensure productive interaction with FcγRs and prevention of anaphylaxis.

Glycans play an important role in IgG-mediated immunity, and crucially IgG-based therapeutics typically have glycan attributes that influence the interaction with FcγRs and downstream immune response.[7–10] Therefore, glycans are important factors in the design of IgG-based therapeutics, particularly in the Fc region, which mediates the effector responses induced by IgG, as well as recycling and the anti-inflammatory activity of IgG.[2,11,12] Currently,

the most important of these appears to be the α(1,6)-linked core fucose, which has been the subject of intensive pharmaceutical interest since it was discovered that IgG lacking this glycan characteristic had enhanced binding to activating FcγRs and improved antibody-dependent cell cytotoxicity (ADCC).[13–18] The market approval of the glycoengineered form of the anti-CD20 Mab Gazyra (Genentech, San Francisco, CA, USA) with reduced core fucosylation highlights the success of this strategy (comprehensive reviews on the biopharmaceutical and therapeutic antibody markets are discussed by Walsh[19] and Ecker et al[20]). Terminal sialylation and mannosylation of antibody N-glycans are also important functional features of antibodies, which significantly impact their activity and serum retention. A high sialic acid content has been proposed to impact the IgG Fc structure and force it to acquire a closed conformation resulting in decreased binding to FcγRs; however, X-ray crystallographic data suggest that this is not the case, and no major Fc structural alterations were observed with increased sialylation.[21,22] Sialylation can also impact the clearance rates of therapeutic antibodies with higher sialylation leading to longer serum retention times[23,24]

and induction of the anti-inflammatory effects of intravenous immunoglobulin (IVIg).[12,25] Terminal mannosylation, usually in the form of hypogalactosylated glycans (G0, G1), can also affect the serum retention of antibodies and binding to mannose-binding lectin (MBL) on macrophages.[26,27] The glycan attributes of potential Mab therapeutics must therefore be carefully considered as the binding to cellular FcγRs, activation of the complement cascade and phagocytosis, serum retention, recycling and placental transport of the therapeutic can be greatly influenced by the Fc glycans.

FcγRs: the key to IgG biological activity

Emerging from the success of Mab therapy and glycoengineering is the importance of FcγRs for their success and therapeutic efficacy and the vast complexity in receptor biology. Therapeutically, FcγRs were once utilized solely for analyzing the efficacy and safety of therapeutic Mabs through biophysical binding experiments; however, this is no longer the case and these antibody receptors are now realizing their potential as anti-inflammatory therapies and in autoimmune conditions. IgG antibodies survey and communicate the information sensed to the immune system via interaction with these single-pass transmembrane receptors of the Ig superfamily. The family of receptors that are found almost ubiquitously throughout the body, from myeloid cells to lymphoid and neuronal cells, are broadly characterized into three groups: FcγRI, FcγRII and FcγRIII.[28] Differences exist between the groups of receptors, particularly in their structure, function, glycosylation and affinity for IgG.[28–33] FcγRI, FcγRIII and FcγRIIa are activating receptors and induce effector activities in innate effector cells such as macrophages and natural killer (NK) cells. FcγRIIb is fundamentally different from the other activating receptors and acts as an inhibitory receptor. Signaling through this receptor induces inhibitory signals that decrease the activation/inhibition (A/I) ratio and bring the cells further from the threshold level required for activation.[34]
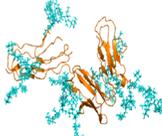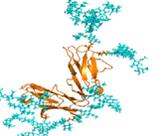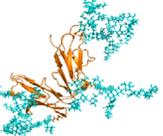
Extensive variability at the genomic, transcriptomic and proteomic level exists among the human FcγRs, with multiple genes, transcripts, polymorphic variants and glycovariants adding to the complexity of these receptors. Polymorphic variants have been found for nearly all of the FcγRs, with significant effects on the interaction with IgG and downstream physiological response.[35–38] Multimerization of antibodies and antigen and engagement of FcγRs lead to microclustering of receptors in the plasma membrane and activation of signaling cascades involving immunoreceptor tyrosine-based activation

motif and immunoreceptor tyrosine-based inhibitory motif resulting in cellular activation or inhibition, activities that can be influenced by the glycosylation state and polymorphic variant of the receptor. It is also important to note that due to the high-affinity nature of FcγRI, it is believed to be constantly bound by monomeric IgG and it is the lower affinity receptor that participates in many of the proinflammatory activities of FcγRs. In addition, when Mabs are used therapeutically, due to the very high serum concentration of IgG (~15 mg/mL), practically all of the cellular FcγRs will be occupied and therefore higher concentrations of a therapeutic antibody are required, further increasing the need for glycoengineered antibodies with higher FcγR affinities than serum IgG.

Glycobiology of FcγRs

Glycosylation research into FcγRs began over 30 years ago, but still currently relatively little is known about how these receptors are glycosylated by cells of the immune system, in healthy and disease states (a review of Fc receptors and glycosylation is discussed by Hayes et al[39]). Several recent structural and biophysical studies have pointed to important roles of FcγR glycosylation and have implicated it in the binding mechanism with IgG.[40–43] The vast majority of glycan data and glycosylation information that is available is for the activating FcγRIIIa receptor, mainly due to its role in NK cell-mediated ADCC and its therapeutic relevance and importance to the pharmaceutical industry. Glycosylation, however, varies widely between the different receptors with different numbers of glycosylation sites and differential and cell type-specific glycosylation patterns (Tables 1 and 2; Figure 2). Seminal work by Edberg et al,[44] Edberg and Kimberley[45] and Kimberly et al[46] showed that FcγRIIIa exists as cell type-specific glycoforms on monocytes and macrophages with different affinities for IgG and different responses to an IgG stimulus due to the differently glycosylated FcγRs. This has intriguing implications for how immune cells respond to IgG based on the glycosylation status of the FcγR. Unfortunately, no further information on the natural glycosylation on these receptors exists in the literature, and there is therefore a lack of information regarding these receptors and the cellular activation or inhibition by IgG. The A/I ratio, for example, is likely to be skewed toward activation or inhibition depending on how a particular cell glycosylates its FcγRs in healthy and disease states. This also has important implications for the biopharmaceutical industry, and response to Mab therapy in patients may depend on how their FcγRs are glycosylated.

Table 1 Properties of human FcγRs

Structure					
Name	FcγRI	FcγRIIa	FcγRIIb	FcγRIIIa	FcγRIIIb
Molecular weight (kDa)	43	35	36	29	26
N-linked sites	7	2	3	5	6
Cells	Dendritic cells Monocytes/macrophages Neutrophils Mast cells	Monocytes/macrophages Langerhans cells Neutrophils Eosinophils Basophils	B-cells/plasma cells Macrophages/monocytes Neutrophils Eosinophils Basophils Langerhans cells Mast cells	Macrophages/monocytes NK cells Mast cells	Neutrophils
Function	High-affinity IgG binding (10^{-9} M Kd) Effector cell activation Phagocytosis	Low-affinity IgG binding (10^{-6} M Kd) Effector cell activation Phagocytosis Degranulation ADCC	Low-affinity IgG binding (10^{-6} M Kd) Inhibition of effector activity	Low-affinity IgG binding (10^{-6} M Kd) Effector cell activation ADCC Phagocytosis	Unknown

Abbreviations: ADCC, antibody-dependent cell cytotoxicity; FcγR, Fc gamma receptor; IgG, immunoglobulin G; NK, natural killer.

Table 2 Annotation of human FcγR glycosylation showing position and conservation of N-glycan sites

| FcγRI numbering	FcγRI	FcγRIIa	FcγRIIb	FcγRIIIa	FcγRIIIb
Domain 1					
52	–	–	–	✓	✓
59	✓	–	–	✓	✓
78	✓	✓	✓	–	✓
88	–	–	–	✓	✓
Domain 2					
152	✓	–	–	–	–
159	✓	✓	✓	–	–
163	✓	–	–	–	–
175	–	–	–	✓	✓
182	–	–	–	✓	✓
Domain 3					
195	✓	na	na	na	na
240	✓	na	na	na	na

Notes: Annotation is based on three-dimensional structures and positions of glycan sites within the three-dimensional structures. The most conserved glycan site across the receptors is Asn 78, which is near the Fc binding site. The Asn 175 glycan site of FcγRIIIa (Asn 162) is almost within the binding site and has been proposed to form carbohydrate–carbohydrate interactions with the Fc glycan. N-glycosylation sites are named for FcγRI using the UniprotKB numbering scheme.
Abbreviation: FcγR, Fc gamma receptor; na, not applicable.

More recently, high-resolution biophysical and structural data collection has revealed the importance of FcγRIIIa glycosylation.[40,43,47] This low-affinity activating receptor is extremely homologous in its extracellular domain, in both amino acid sequence and three-dimensional structure to the related FcγRIIIb.[48,49] Two N-linked sites of FcγRIIIa have been shown to regulate the binding of IgG; a glycan on Asn 45 has an inhibitory role and negative effect on IgG binding, whereas glycosylation at Asn 162, which is located at the IgG-binding interface in the three-dimensional structure, increases IgG interaction and binding affinity[40–42] (Figure 3). Glycan analysis of FcγRIIIa from recombinant systems followed by biophysical binding experiments showed that glycosylation is dependent on the source of the receptor and that specific glycans can be located on the Asn 162 site that influences and mediates IgG binding[43,47,50] (Figure 3). Structural studies have also shown that on a molecular level a unique carbohydrate–carbohydrate interface exists between afucosylated IgG1 and FcγRIIIa, which can explain the increase in affinity for therapeutic antibodies lacking core fucose.[40]

Human immune cells have different combinations of FcγRs with different numbers of N-glycosylation sites (Table 1 and Table 2), and IgG immune complexes will interact with many different receptors on the same cell, creating an extremely complex series of interactions and signaling pathways/stimuli. Adding further complexity is the differential glycosylation of the FcγRs, which are found on the same cell. Previous studies have described the glycan compositions of recombinant FcγRs (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and FcγRIIIb) from different sources and showed that the glycosylation is complex with multi-antennary structures and extensive outer-arm modifications (Figure 2).[43,50–55] FcγRI, which has seven potential N-glycosylation sites, is structurally different from the other receptors with an extra
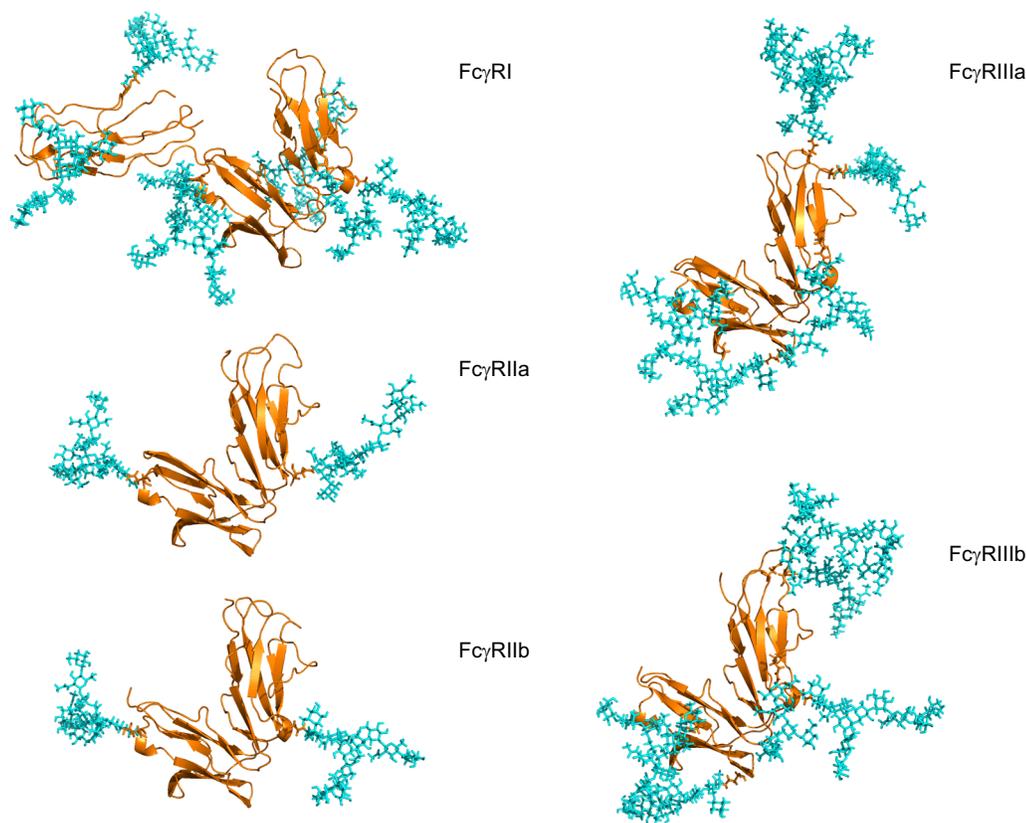
Figure 2 Human FcγRs are complex glycoproteins.

Notes: FcγR ectodomains were modeled with N-glycans on each glycosylation site for each receptor, taking into account glycan size and composition, torsion angles and free energies. The N-glycans (shown in cyan) were modeled based on identified glycans from FcγRs (shown in gold) produced in recombinant systems such as NS0, CHO and HEK293 systems.[43,47,50] In the case of FcγRIIIa, site-specific glycosylation studies were performed by Zeck et al,[47] and this information was used to build the glycans shown on the Asn 162 site located at the binding interface with IgG. For the remaining receptors, no site-specific analysis was available, and in these cases the most abundant glycans identified from the recombinant sources were used to model N-glycosylation for these receptors. FcγRI has an extra D3 domain, which contributes to its high-affinity nature, and this domain also contains two glycosylation sites. The glycan compositions modeled onto each N-glycosylation site for each FcγR are named according to the Oxford notation (https://glycobase.nibrt.ie/glycobase/show_nibrt.action)[86] and are as follows: FcγRI: Asn 59 (Man 5), Asn 78 (FA2G2S1), Asn 152 (FA2GN2S2), Asn 159 (Man 6), Asn 163 (FA2G2), Asn 195 (FA2G1GN1), Asn 240 (FA2BG2). FcγRIIa: Asn 64 (FA2G2S1), Asn 145 (FA2BG2). FcγRIIb: Asn 66 (FA2G2S1), Asn 147 (FA2BG2). FcγRIIIa: Asn 38 (FA2G2S1), Asn 45 (FA2G2), Asn 74 (FA4G4S4), Asn 162 (FA2G2), Asn 169 (FA2BG2). FcγRIIIb: Asn 35 (FA2GalNAc2S2), Asn 42 (Man 5), Asn 61 (FA2G2S1), Asn 71 (FA3G2), Asn 159 (FA2BG1), Asn 166 (FA2BG2). PDB accession numbers used to build the models were as follows: FcγRI: 4x4m, FcγRIIa: 1fcg, FcγRIIb: 2fcb, FcγRIIIa: 3ay4, FcγRIIIb: 1e4j.

Abbreviations: FcγR, Fc gamma receptor; IgG, immunoglobulin G; PDB, Protein Data Bank.

D3 domain, which contributes to its high-affinity nature.[56,57] There is little information available regarding the nature of FcγRI glycosylation or the glycosylation site occupancy; however, studies performed on recombinant FcγRI from NS0 and HEK293 cells showed that the receptor expressed significant amounts of high-mannose glycans and complex multi-antennary structures with large amounts of core-fucosylation and outer-arm modifications; glycan monosaccharide compositions which can influence the IgG-binding interaction (Figures 3 and 4).[43,50] Crystal structures of FcγRI are available, and recently a crystal structure in complex with IgG was described (Figure 3); however, even though the IgG glycans were shown to be important for the interaction, little information is available on the receptor glycans (Figures 3 and 4).[58,59] Biologically and functionally FcγRIIa and FcγRIIb are significantly different as FcγRIIb is the inhibitory FcγR;

however, despite the biological differences, they demonstrate significant homology in their extracellular domains with ~92% sequence identity. Data exist for the glycosylation of the receptors from NS0, HEK293, CHO and insect cells and demonstrate that the receptors display complex glycan structures with core fucosylation and minimal sialylation (Figure 2).[43,50–52] In one report of glycosylation of FcγRIIa from insect cells, sialylation was not detected and glycosylation was not reported to influence IgG binding.[51] The only information on FcγRIIb glycosylation comes from reports describing recombinant sources from NS0 and HEK293 cells, and similar to other FcγRs this receptor presents with multi-antennary structures, which are core fucosylated and undersialylated (Figure 2).[43,50] FcγRs have a number of glycan characteristics in common, all contain complex multi-antennary structures, which are core fucosylated and
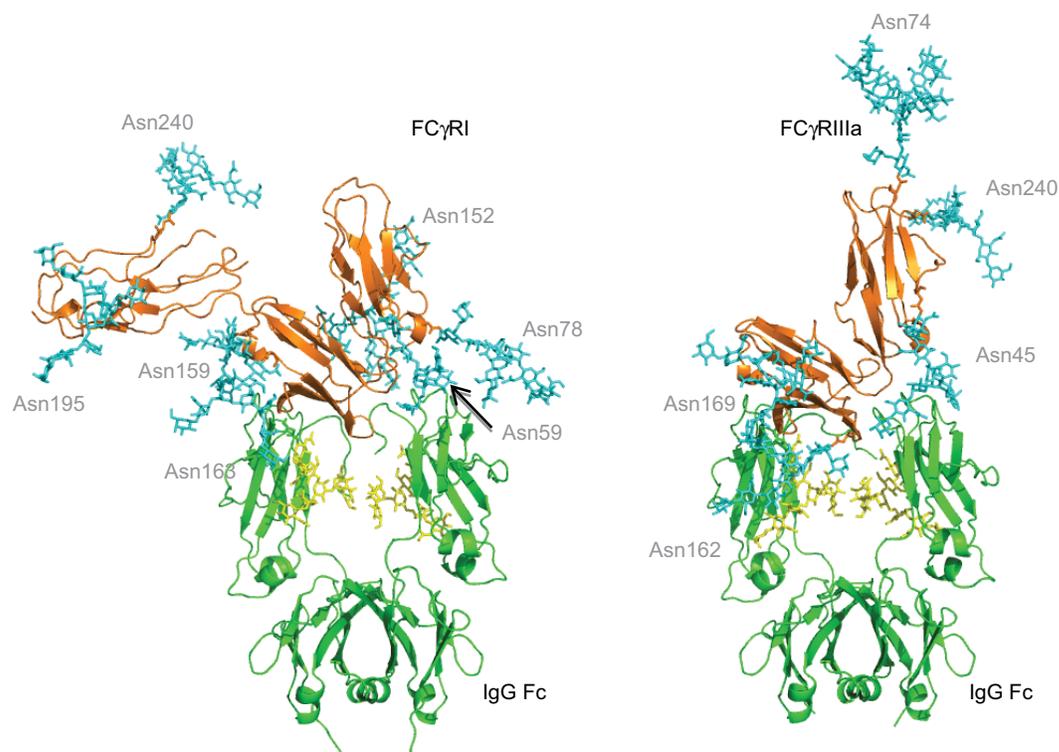
Figure 3 FcγR–IgG complexes with modeled N-glycans show complexity of glycosylation and the potential roles of glycans in the binding interaction with IgG.
Notes: The Asn 162 glycan of FcγRIIIa has been shown to form carbohydrate–carbohydrate interactions with the bi-antennary glycan of IgG.[40] This asparagine residue is at the binding interface with IgG. FcγRI does not have a glycan in this position but does have glycans near the binding site such as Asn 78 (Asn 162 in FcγRIIIa), which is structurally conserved in each of the FcγRs. The glycan compositions modeled onto each N-glycosylation site for each FcγR are named according to the oxford notation (see https://glycobase.nibrt.ie/glycobase/show_nibrt.action)[86] and are as follows: FcγRI: Asn 59 (Man 5), Asn 78 (FA2G2S1), Asn 152 (FA2GN2S2), Asn 159 (Man 6), Asn 163 (FA2G2), Asn 195 (FA2G1GN1), Asn 240 (FA2BG2). FcγRIIIa: Asn 38 (FA2G2S1), Asn 45 (FA2G2), Asn 74 (FA4G4S4), Asn 162 (FA2G2), Asn 169 (FA2BG2). PDB accession numbers used to build the models were as follows: FcγRI: 4x4m, FcγRIIIa: 3ay4.
Abbreviations: FcγR, Fc gamma receptor; IgG, immunoglobulin G.
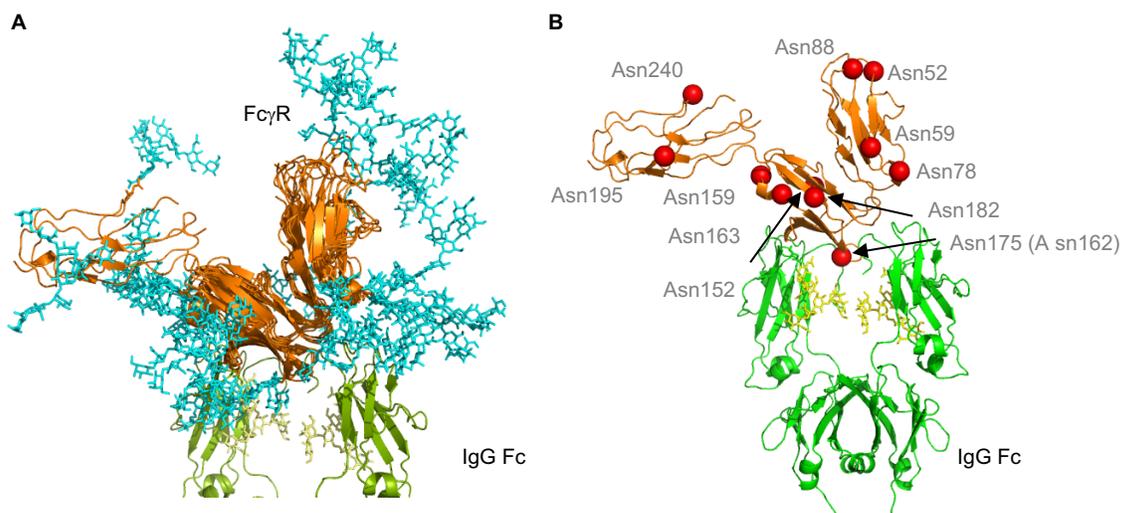
Figure 4 Human FcγRs show complex N-glycosylation in the protein ectodomain and around the IgG binding site.
Notes: (**A**) Structural overlay of FcγRs. FcγRs are very structurally homologous with the exception of the extra D3 domain in FcγRI. Complexity of glycans (cyan) is shown and potential interactions with IgG Fc and the IgG Fc glycans. The glycan compositions modeled onto each N-glycosylation site for each FcγR are named according to the oxford notation (see https://glycobase.nibrt.ie/glycobase/show_nibrt.action)[86] and are as follows: FcγRI: Asn 59 (Man 5), Asn 78 (FA2G2S1), Asn 152 (FA2GN2S2), Asn 159 (Man 6), Asn 163 (FA2G2), Asn 195 (FA2G1GN1), Asn 240 (FA2BG2). FcγRIIa: Asn 64 (FA2G2S1), Asn 145 (FA2BG2). FcγRIIb: Asn 66 (FA2G2S1), Asn 147 (FA2BG2). FcγRIIIa: Asn 38 (FA2G2S1), Asn 45 (FA2G2), Asn 74 (FA4G4S4), Asn 162 (FA2G2), Asn 169 (FA2BG2). FcγRIIIb: Asn 35 (FA2GalNAc2S2), Asn 42 (Man 5), Asn 61 (FA2G2S1), Asn 71 (FA3G2), Asn 159 (FA2BG1), Asn 166 (FA2BG2). PDB accession numbers used to build the models were as follows: FcγRI: 4x4m, FcγRIIa: 1fcg, FcγRIIb: 2fcb, FcγRIIIa: 3ay4, FcγRIIIb: 1e4j. (**B**) Position of N-glycan sites in human FcγRs. Asn 175 (Asn 162 in FcγRIIIa) is at the binding site with IgG and glycans in this position can participate in carbohydrate–carbohydrate interactions. A number of other glycan sites are present close to the binding interface with IgG and glycans in this position can potentially participate in glycan–glycan interactions and glycan–protein interactions with IgG Fc. Structure is based on FcγRI (PDB: 4x4m). N-glycosylation sites are named for FcγRI using the UniprotKB numbering scheme.
Abbreviations: FcγR, Fc gamma receptor; IgG, immunoglobulin G; PDB, Protein Data Bank.

undersialylated (Figure 2). Oligomannose structures are also present and vary depending on the receptor with the largest amount observed for FcγRI (Figure 2). Very little information exists as to the natural glycosylation state of any of the receptors in healthy or disease states, and little information exists as to the glycosylation site occupancy for any of the natural receptors and only for recombinant FcγRIIIa.[47]

FcγRs as therapeutic targets and a role for glycosylation

FcγRs mediate many of the biological functions of therapeutic Mabs, particularly when the induction of effector activities is desired. Manipulating the Fc glycan of Mabs is a successful strategy to prevent interaction with FcγRs when the activation of the immune system is not required for the efficacy of the antibody or is undesirable. In the primary mechanism of oncology Mabs such as rituximab and trastuzumab, FcγRIIIa is targeted to induce ADCC. Glycoengineering of the antibody Fc region has proved to be a highly successful strategy to target and improve FcγR binding and ADCC, and the next-generation glycoengineered afucosylated Mab Gazyva is now on the market. It is also worth noting that aglycosylated IgG variants with specific mutations in the Fc region have been shown to bind FcγRI with equal or greater affinity than wild-type IgG, presenting an alternative therapeutic strategy.[60] Various glycosylation modeling platforms have also added to our understanding of complex networks leading to specific glycoforms.[61–65] Polymorphisms found in the extracellular domain of FcγRs further add to their variability and complexity, in particular, the Val 158/Phe 158 polymorphism of FcγRIIIa and Arg 131/His 131 polymorphism of FcγRIIa dictate how a patient responds to antibody therapy with the Val 158 and His 131 variants responding better to rituximab treatment in non-Hodgkins lymphoma.[66,67] Furthermore, with detailed knowledge of the glycosylation of FcγRs in patients, there is the potential to manipulate and target an FcγR glycoprofile to improve the therapeutic effect. Prediction of how a patient will respond to antibody therapy or identification of biomarkers for nonresponders are important factors for developing a personalized medicine approach.

FcγR expression and regulation are important factors in antibody therapy for a range of clinical conditions. Receptor expression levels are shown to differ in patients with cancer, and the inhibitory FcγRIIb is shown to be upregulated in conditions such as malignant melanoma and lymphomas.[68–71] Inhibitory receptor expression is also reported to be decreased on memory B-cells and plasma cells from patients with chronic inflammatory demyelinating

neuropathy treated with IVIg, suggesting that in inflammatory or pro-inflammatory conditions FcγR activation prevails or is increased over inhibitory conditions.[72] In inflammatory bowel disease and systemic lupus erythematosus, conditions characterized by chronic inflammation, FcγRI upregulation has been reported.[73,74] Higher expression levels of FcγRIIa and FcγRIIIa have also been reported in autoimmune conditions, and the anti-TNF Mab infliximab has been reported to decrease the expression of activating receptors.[75] Viral and bacterial infections also influence the surface numbers of activating and inhibitory FcγRs, and bacterial components such as lipopolysaccharide increase the expression of FcγRIII and FcγRIV in mice[76] and cytokines such as interferon-γ can regulate or alter FcγR expression, particularly in viral infections such as HIV.[77,78] In these cases, an antibody or combination therapy to block a particular receptor such as the inhibitory receptor in cancer or activating receptors in inflammation could prove to be an effective strategy by skewing the A/I ratio toward cellular activation or inhibition. Furthermore, there are many examples of cancer-specific glycosylation changes, which promote metastasis, survival and immune evasion. It is therefore likely that the glycosylation of FcγRs present on cancer cells such as non-Hodgkin lymphoma will be affected in a way that will prevent productive antibody interactions. It is also likely that cancer cells will negatively influence the glycosylation of FcγRs on cytotoxic cells, such as NK cells to inhibit productive antibody interactions and promote cancer cell survival. Immunotherapies can be improved with the knowledge of the glycosylation profiles of FcγRs in healthy and disease states.

Glycosylated FcγRs as therapeutics

Targeting FcγRs with small molecule inhibitors or anti-receptor Mabs is an attractive strategy to prevent immune complex-driven activation of effector cells, a major driver of inflammation and autoimmunity. Since the 1990s, soluble FcγRs, formed by alternate splicing or proteolytic cleavage of the receptor ectodomain, have been identified in humans and mice and have been shown to inhibit B-cell proliferation and IgG production.[79] Recombinant forms of the soluble ectodomains of activating receptors (FcγRI, FcγRII and FcγRIII) and more recently the inhibitory FcγRIIb have been used to perform a similar therapeutic anti-inflammatory role in humans with significant success.[80–84] These soluble domains are believed to function as decoy receptors to bind IgG immune complexes, decrease the A/I ratio and prevent inflammation and autoimmunity.[85] Although these soluble FcγRs have low affinity (except FcγRI) for IgG immune

complexes, positive results in reducing inflammation have been shown in epidermolysis bullosa acquisita.[80,84] In addition to the low-affinity nature of soluble FcγRs, another downside to their use as therapeutics is their relatively small size, ranging from 20 to 45 kDa in their aglycosylated state (Table 1). This leads to difficulties such as rapid excretion in vivo. A possible mechanism to increase the size of the FcγR therapeutic is to use recombinant glycosylated forms made in cells such as CHO cells or HEK293 cells (Figure 2), as opposed to aglycosylated forms made in bacteria such as *Escherichia coli*. In addition to increasing the size of the therapeutic, the strategy of using glycosylated ectodomains has the added benefit of increasing the solubility of the receptor and may in addition help in the clearance of glycosylated FcγR immune complexes through interactions with lectins such as MBL on macrophages through phagocytosis. Glycosylated FcγR ectodomains have the potential to increase the clearance of immune complexes and further reduce inflammation through this glycosylated FcγR–lectin-based phagocytosis mechanism.

Glycosylation of FcγRs: therapeutic prospects

There is now clear evidence that FcγR glycosylation is an important factor in the interaction with therapeutic antibodies and could influence immune system activation or inhibition.[40,43,47] This has important implications for how Mab-based therapeutics and fusion proteins are designed. However, little is known about the glycosylation of these receptors in their natural environment and until detailed information on how each receptor is glycosylated by different cells of the immune system in healthy and disease states is available, complete understanding of how therapeutic antibodies interact with the immune system to activate or inhibit will remain incomplete. Although important information is available, which shows that immune cells such as macrophages and monocytes bind and respond to IgG differentially, most of the detailed information on glycosylation and its influence on the IgG–FcγR interaction come from recombinant systems and receptors expressed in NS0, HEK293 and CHO cells.[43–47] This information is valuable and shows that receptor glycosylation is cell type specific and influences the IgG-binding kinetics and indicates that cells of the immune system such as NK cells, macrophages, neutrophils and B-cells will also glycosylate in a cell type-specific manner, which can result in each cell responding differently to IgG and IgG immune complexes. The particular glycans that the cell expresses on the FcγR, based on its own glycosylation machinery, can determine the antibody response and it is conceivable that in a state of

inhibition or inactivation specific glycans can be expressed on the FcγR that can prevent a productive interaction with immune complexes or decrease the affinity for antibody. Conversely, in a state of inflammation or activation, different glycans can be expressed on the FcγR to promote a positive antibody interaction and induce effector responses. FcγR glycosylation can therefore be used as a mechanism by the immune system to fine-tune the antibody response. In inflammatory and autoimmune conditions, for example, FcγRs found on macrophages, monocytes and neutrophils are potentially glycosylated in a manner that facilitates excessive interaction with antibodies, immune complexes or self-antigens that promote inflammation and autoimmunity. In addition, in healthy states, glycans can potentially be used by FcγRs to inhibit or decrease antibody engagement, downregulate immune responses and prevent inflammation and autoimmunity. Glycans on FcγRs can therefore be used to alter the balance between activation and inhibition in a glycosylation-mediated control mechanism. In addition, until this detailed glycan information is available, the glycosylation of FcγRs cannot be fully understood with the aim of manipulating or optimizing therapeutics based on the glycosylation state of the FcγR. There is the potential to design therapeutics to specifically target known FcγR glycoforms in a particular disease such as cancer or inflammatory or autoimmune conditions, which are known to promote or inhibit productive antibody interactions. Information exists that monocyte FcγRIIIa does not contain high-mannose-type glycans whereas NK cell FcγRIIIa does, and this can explain the lower affinity of the monocyte/macrophage glycoform.[45] This information, together with further detailed glycan data, can be exploited to design therapeutics to differentially bind specific FcγR glycoforms such as an Mab with higher affinity for the high-mannose-type glycoform of macrophages if antibody-dependent phagocytosis is desired.

Detailed knowledge of FcγR glycobiology in inflammatory and autoimmune conditions and the availability of glycan information will allow for a deeper and more comprehensive knowledge and understanding of these conditions and how to treat them, the type of antibody isotype, glycoform or fusion protein to design and a much more targeted approach to an individual or condition. This raises the possibility of a personalized medicine approach, whereby patient's FcγR glycoforms could be determined and the therapeutic approach tailored to suit a particular person. A patient's FcγRs, including polymorphisms and glycoprofiles, could be used to predict the efficacy of a therapeutic Mab and identify responders and nonresponders to expensive and potentially dangerous biological therapies. There is evidence in the literature that

the glycans present on FcγRs made in recombinant systems can modulate the binding interaction with antibody.[43] By knowing the natural glycoprofiles of these receptors, the interaction of therapeutic Mabs with their target cells such as NK cells in cancer treatment can be better understood and the clinical outcome can be better predicted.

Finally, the biopharmaceutical industry typically uses biophysical techniques such as surface plasmon resonance and recombinant forms of FcγRs expressed in NS0, HEK293 or CHO cells to determine the interactions of therapeutic antibodies with FcγRs, affinity constants and kinetic parameters. While these systems provide valuable information, the downside to this approach is that the physiological system is not fully represented and these recombinant receptors do not adequately predict the outcome of an antibody therapy or the interactions of a therapeutic antibody with cells and FcγRs of the immune system, partly because the FcγRs used in these analyses are glycosylated differently to the FcγRs of the immune system. There are examples in the literature, which show that the interaction kinetics of therapeutic antibodies is different in biophysical experiments depending on the source of the receptor and its glycosylation pattern.[47,50] By understanding the glycosylation of the natural FcγRs, there is the potential to design and use recombinant forms of the receptors, which have glycoprofiles that physiologically resemble the natural FcγRs in biophysical evaluations to provide more accurate affinity determinations, analysis of the interaction and ultimately predictions of the physiological outcome of the antibody therapy.

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Disclosure

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