Glycosylation and Fc Receptors

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Abstract  Immunoglobulins and Fc receptors are critical glycoprotein components of the immune system. Fc receptors bind the Fc (effector) region of antibody molecules and communicate information within the innate and adaptive immune systems. Glycosylation of antibodies, particularly in the Fc region of IgG, has been extensively studied in health and disease. The N-glycans in the identical heavy chains have been shown to be critical for maintaining structural integrity, communication with the Fc receptor and the downstream immunological response.
Less is known about glycosylation of the Fc receptor in either healthy or disease states, however, recent studies have implicated an active role for receptor associated oligosaccharides in the antibody-receptor interaction. Research into Fc receptor glycosylation is increasing rapidly, where Fc receptors are routinely used to analyze the binding of therapeutic monoclonal antibodies and where glycosylation of receptors expressed by cells of the immune system could potentially be used to mediate and control the differential binding of immunoglobulins. Here we discuss the glycosylation of immunoglobulin antibodies (IgA, IgE, IgG) and the Fc receptors (FcαR, FcεR, FcγR, FcRn) that bind them, the function of carbohydrates in the immune response and recent advances in our understanding of these critical glycoproteins.

**Keywords** Immunoglobulin • Fc receptor • Glycosylation • Immunity • N-glycan • O-glycan

**Abbreviations**

- α-gal: Gal(α1,3)-gal
- ADCC: Antibody dependent cell mediated cytotoxicity
- Asn: Asparagine
- CDC: Complement mediated cytotoxicity
- DC-SIGN: Dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin
- FcαR: Fc alpha receptor
- FcεR: Fc epsilon receptor
- FcγR: Fc gamma receptor
- FcRn: Neonatal Fc receptor
- GALT: Gut associated lymphoid tissue
- GlcNAc: N-acetylg glucosamine
- Ig: Immunoglobulin
- IgA: Immunoglobulin A
- SIgA: Secretory IgA
- IgE: Immunoglobulin E
- IgD: Immunoglobulin D
- IgG: Immunoglobulin G
- IgM: Immunoglobulin M
- IgSF: Immunoglobulin-like super family
- ITAM: Immunotyrosine-like activation motif
- ITIM: Immunotyrosine-like inhibition motif
- Man: Mannose
- MBL: Mannan binding lectin
- MMR: Macrophage mannose receptor
- MHC: Major histocompatibility complex
- NGNA: N-glycolylneuraminic acid
1 Introduction

Glycosylation is a key player in the immune response, in antigen recognition of invading microorganisms and in ligand–receptor interactions that lead to cellular and effector activities. Modification of proteins, lipids, and other organic molecules by glycosylation creates a repertoire of glycovariants, some of which may confer orthogonal functions on the proteins to which they are attached. Given the diversity and ubiquity of glycan structures in almost all organisms it is not surprising that the immune system has evolved to detect sugar epitopes from pathogenic sources. Toll-like receptors (TLR) are a particularly good example of this pathogen associated molecular pattern (PAMP) recognition. Endotoxin (lipopolysaccharide), an outer membrane component of gram negative bacteria consisting of a polysaccharide and lipid is sensed by TLR4 which signals to activate the innate immune system (Takeda et al. 2003; Medzhitov et al. 1997; Chow et al. 1999). Mannan binding lectin (MBL) is a C-type lectin that functions in pattern recognition of sugars from pathogenic micro-organisms leading to activation of the lectin pathway and complement system (Petersen et al. 2001). Macrophage mannose receptor (MMR) and dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin...
(DC-SIGN) are also C-type lectins which recognize mannose type sugars on bacteria, viruses and fungi (Figdor et al. 2002). Galectins are widely distributed proteins that bind galactose and its derivatives and function in cell–cell and cell–matrix interactions and cell signaling (Barondes et al. 1994a, b). Dectin-1 is another C-type lectin, which mediates various immune functions in response to fungal infection (Brown and Gordon 2001; Ariizumi et al. 2000). This carbohydrate recognition of pathogenic microorganisms by specific sugar binding proteins of the immune system is a critical sensing and defense mechanism. Effective immunity also requires the ability of cells to communicate with each other, to extravasate from blood vessels to sites of infection and to fight the source of infection and maintain normal cellular homeostasis, critical functions mediated by carbohydrates.

Immunoglobulins and their receptors (Fc receptors) are critical glycoprotein components of the immune system that link the innate and adaptive arms of immunity. Immunoglobulins are diverse glycoproteins in terms of their isotype, antigen recognition, distribution/concentration, and effector responses. They can be membrane bound in the form of surface immunoglobulins or soluble. Surface immunoglobulins form part of the B-cell receptor (BCR) and are composed of membrane bound immunoglobulin D (IgD) or immunoglobulin M (IgM). This allows the antibody producing B-cell to detect specific antigens resulting in B-cell activation and antibody production. In humans, there are five types of immunoglobulin antibody: IgA, IgD, IgE, IgG, and IgM, each with specific structure, function and activities. Each antibody monomer consists of two identical heavy chains and two light chains, an Fc domain formed from the constant regions and two antigen binding Fab domains formed from the variable regions but different antibodies have variable numbers of immunoglobulin domains. Somatic hypermutation in the variable region allows antibodies to recognize a huge repertoire \((10^9)\) of antigens and the constant Fc region allows the antibody to interact with Fc receptors on the surface of innate immune cells such as monocytes, neutrophils, B-cells, macrophages and natural killer (NK) cells. The N-linked glycans present on conserved asparagine containing sequons in the Fc region, particularly IgG are essential for antibody structure and function.

Antibodies communicate with effector cells of the immune system through interactions with membrane bound Fc receptors, which are complex glycoproteins found on many cell types. These interactions induce effector responses including phagocytosis, activation of the complement cascade, and antibody dependent cell-mediated cytotoxicity (ADCC). Even though IgG antibodies bind to complement receptors and C-type lectins for the purpose of this review we focus on the interactions of immunoglobulins with Fc receptors. Fc receptors \((F_{\gamma}C_R, F_{c\alpha}R, F_{c\gamma}R)\) are specific for a particular antibody isotype and direct specific immune responses following binding of antibody-immune complexes. Fc receptors have distinct functions but similar structures with familiar domains, architecture and post-translational modifications (see Fig. 1). The IgD receptor is found on B-cells and the recently discovered receptor for IgM \((F_{c\mu}R)\) is expressed only in lymphoid cells such as B- and T-lymphocytes in humans (Kubagawa et al. 2009; Ouchida et al. 2012). There is an additional Fc receptor known as the neonatal Fc receptor
(FcRn), which is involved in recycling of IgG, maintaining the serum concentrations of the antibody where high concentrations are required to fight infection (Ward et al. 2003). This receptor is also involved in placental transport of IgG from mother to fetus and is critically important for the transfer of humeral immunity to the baby, which cannot yet make IgG antibodies (Morphis and Gitlin 2003).
Glycosylation in antibody-receptor interactions has traditionally focused on the function of the N-linked glycans of immunoglobulins, in particular IgG, however, recent exciting discoveries have led to renewed interest and focus on the role of Fc receptor glycosylation in the antibody interaction and subsequent immune response.

Much of the available glycosylation data and information on antibodies and Fc receptors come from studies involving IgG and FcγRs. For this reason, in this chapter we focus mainly on this particular antibody isotype and Fc receptor class. We have chosen not to discuss in any detail IgD or IgM and the corresponding receptors, as there is little glycosylation data available for these receptors. In addition to IgG and FcγRs, we also discuss the extensive glycosylation of IgA and IgE and their receptors: FcζR and FcεR. Where data are available, we discuss the influence of glycosylation on the antibody interactions and immune response. The biopharmaceutical industry is particularly interested in the crucial influence of particular glycans, such as noncore-fucosylated structures, attached to therapeutic monoclonal antibodies. An explosion of genomic, proteomic, and structural data now exists and atomic resolution structures of antibody-receptor complexes are available, which shed light on the mechanisms of interaction and the detailed influence of the associated sugars. We discuss these structures and the ways in which carbohydrates are involved in unique carbohydrate–carbohydrate and carbohydrate–protein interactions.

2 IgA-Mediated Immunity

Antibody-mediated innate and adaptive immunity at mucosal membranes and secretions is largely facilitated by the activity of immunoglobulin A (IgA and secretory IgA), an important class of the immunoglobulin family that provides protection against pathogens at mucosal sites such as the gastrointestinal, genitourinary, and respiratory tracts (Macpherson et al. 2000). IgA comprises approximately 15 % of total body immunoglobulin and, in adults, is produced by the majority of plasma B-cells in larger amounts than all of the other immunoglobulins combined (66 mg/kg/day) (Kerr 1990). IgA, which is unlike other immunoglobulins in that it forms a T-shaped structure exists in a number of forms or subclasses (see Fig. 2a) (Boehm et al. 1999; Furtado et al. 2004). Serum IgA1 and IgA2 which differ by a thirteen amino acid sequence found in the hinge region of IgA1 (van Egmond et al. 2001). This additional hinge region is heavily O-glycosylated, protecting the antibody from bacterial proteolytic degradation (see Fig. 2a). IgA2 does not have these O-linked sites but does contain two additional N-linked sites making it less susceptible to bacterial proteolysis. Polymeric forms of IgA also exist, composed of 2–4 IgA monomers joined by a 16 kDa chain (J chain) (Kerr 1990). This form of IgA, which is found in secretory fluids such as saliva, tears, colostrum, and gastrointestinal fluids, is known as secretory IgA (SIgA). At mucosal sites such as the gastrointestinal tract, IgA exists...
Fig. 2 Immunoglobulin A, glycosylation and the FcαR complex. 

(a) Schematic representation and molecular model of human IgA1 showing glycan sites and glycan site occupancy. The hinge region of IgA is heavily O- and N-glycosylated which protects IgA from bacterial proteolysis. Also shown is Fc glycosylation and extra C-terminal tail region glycosylation containing bi-antennary glycans. 

(b) IgA1–FcαRI complex. Two FcαR molecules are bound to the IgA antibody in the crystal structure. FcαR glycosylation and site occupancy is shown in red and IgA glycosylation is shown in blue. IgA glycans (blue) appear on the external side of the Cα2 domain of FcαRI. It is believed that the Fcα glycan of IgA comes within 8 Å of the FcαR, indicating a potential protein–carbohydrate or carbohydrate–carbohydrate interaction with the glycans of FcαRI (red). The model is based on 1ow0 in the protein data bank.
primarily as SIgA and exhibits anti-inflammatory neutralizing properties. This prevents unwanted immune responses against commensal bacteria or other perceived antigens encountered in a normal diet.

SIgA is extensively N- and O-linked glycosylated (see Fig. 2). Both IgA1 and IgA2 contain two N-linked sites per H chain, one in the Cα2 domain (Asn263) and one in the C-terminal region (Asn459). The secretory component (SC) is also heavily glycosylated with seven N-linked sites and an additional site in the J chain. The IgA1 hinge region is heavily O-glycosylated. Comprehensive analysis of the N- and O-linked glycans of IgA were described in several reports which identified significant heterogeneity in both the N- and O-linked forms (Pierce-Cretel et al. 1981, 1982, 1989; Mizoguchi et al. 1982; Hughes et al. 1999). The total glycosylation of SIgA was first reported by Royle et al. in (2003) from the antibody H, J and SC peptide chains from pooled human serum IgA. A large variety of N- and O-linked carbohydrate epitopes were described in the SC and hinge region of the H chain respectively such as galactose in β(1,3) and β(1,4) linkage to GlcNAc, fucose in α(1,2) linkage to galactose and α(1,3) and α(1,4) linkage to GlcNAc and α(2,3) and α(2,6)-linked sialic acids (Royle et al. 2003). An abundance of sia
glycosylated N-linked structures in the SC were identified with shorter truncated glycans within the heavy chain region. Notably, all of the Lewis and sial-Lewis glycan epitopes were identified conferring potential binding sites for lectins and bacterial adhesins. These carbohydrate epitopes serve a dual role in protecting SIgA from bacterial proteolysis and in ligand binding. In addition, SIgA glycans facilitate binding to the lectin Mac-1(CD11b/CD18), which plays a role in FcαR-mediated SIgA signaling (van Spriel et al. 2002). Glycosylation of the Fab regions of IgA was reported by Mattu et al. (1998). Bi-antennary N-linked glycans were mainly present in the Fc whilst N- and O-linked sugars with extensive sialylation (30%) were reported for the Fab regions (Matti et al. 1998).

Serum IgA, which is present throughout the body following its synthesis by plasma cells in the spleen, is distinct from SIgA which is present at secretory sites such as gut associated lymphoid tissue (GALT). Glycosylation of the various forms of IgA is also different, probably due to the differences in the 3D structures of the individual proteins and the levels of the glycan processing enzymes at their sites of production. Polymeric SIgA has several binding sites for antigen and due to the differences in carbohydrate composition such as exposed GlcNAc and mannose residues it can be internalized by dendritic cells following binding to mannose receptor, whereas serum IgA cannot (Royle et al. 2003; Heystek et al. 2002). Large, complex, sialylated O-glycans in the hinge region differ between serum IgA and SIgA and are also likely to play different roles in interactions with bacterial adhesins. Interestingly, Royle et al. show that N-glycans on the H chains of SIgA contain exposed mannose and GlcNAc residues that can be masked by the SC (Royle et al. 2003). Disruption of the SC–H chain can reveal these exposed terminal monosaccharides, which can then be recognized by lectin receptors on dendritic cells and phagocytes to promote opsonisation and phagocytosis.
2.1 FcαR

IgA antibodies communicate with the immune system via interaction with FcαRs, integral membrane proteins specific for the Fc region of the IgA molecule (see Fig. 2b). Five structurally unrelated FcαRs have been described (Monteiro and van de Winkel 2003). The polymeric IgA receptor (pIgR) is a member of the immunoglobulin superfamily, which binds dimeric SIgA and is expressed by mucosal epithelial cells. Following SIgA binding, translocation of the IgA–pIgR receptor complex across the epithelial cell delivers the antibody to the mucosal surface (Mostov 1994). At the mucosal surface, the SC (part of the epithelial cell) is cleaved and the remaining complex (IgA–J chain–SC) is secreted. Interestingly, this peptide complex is assembled from two different cell types and represents one of the few examples where a protein is assembled from two distinct cell types. The polymeric IgA receptor also facilitates the secretion of IgM antibodies (Johansen et al. 1999).

FcαRI (CD89) is a specific Fc receptor for IgA1 and IgA2 found on cells of myeloid lineage such as macrophages, neutrophils, dendritic cells, and eosinophils (Geissmann et al. 2001; van Egmond et al. 2000; Monteiro et al. 1993). Due to alternative splicing, the FcαRI protein can exist in three different forms or splice variants in vivo (Morton et al. 1996; Pleass et al. 1996). The FcαRI (a.1) isoform is the full-length protein and is a 32 kDa single pass transmembrane receptor (Maliszewski et al. 1990). However, due to extensive glycosylation on six potential N-linked sites and seven potential O-linked sites and further heterogeneity which exists on these glycosylation sites the mature protein exists with a molecular weight of between 50 and 100 kDa in vivo (Morton et al. 1996). FcαRI can range in mass from between 55 and 75 kDa in the case of monocytes and neutrophils to as high as 100 kDa in the case of eosinophils due to differential glycosylation (Morton et al. 1996; van Egmond et al. 2001). Little information exists as to the exact nature of the glycans found on FcαRI, however, deglycosylation experiments using endoglycosidases confirmed the presence of extensive glycosylation and also suggested that FcαRI was differentially glycosylated. All of the six potential N-linked sites have been the focus of recent investigations. Site-directed mutagenesis of each N-linked site revealed no impact on FcαRI binding of IgA, with the exception of Asn58 (Xue et al. 2010). Mutagenesis of Asn58 to Glu58 resulted in a near twofold increase in binding of IgA. This study also investigated the role of sialylation on IgA binding and demonstrated a near fourfold increase in affinity following neuraminidase treatment of mutagenized FcαR and provided a clear indication that glycosylation of FcαR at position 58 is a key factor in the binding affinity for IgA. The full role of FcαR glycosylation is currently unclear but these recent experiments indicate that different glycans play a role in the IgA interaction and that particular cell types glycosylate the receptor in a cell-type specific manner. It remains a challenge to characterize FcαR glycosylation, define its role and mechanism in binding IgA and control of downstream immune responses.
Serum IgA principally exists as a monomer and interacts with FcεRI leading to immune effector responses (see Fig. 2b). The precise role of FcεR, however, is still debated. FcεRI exists as a low affinity Fc receptor for IgA with an approximate \( K_a = 10^6 \text{ M}^{-1} \) that rapidly dissociates from the FcεRI–IgA complex (Wines et al. 1999). In common with IgG binding to Fcγ receptors, IgA immune complexes bind more tightly to FcεR and with higher avidity than monomeric IgA (van Egmond et al. 2001). Soluble glycosylated forms of FcεRI also exist with molecular weights of approximately 30 kDa (25 kDa peptide sequence) and 50–70 kDa. The latter tightly associates with polymeric IgA, however, the function of soluble FcεRI is unclear (van Zandbergen et al. 1999; van der Boog et al. 2002). The crystal structure of the FcεR–IgA complex indicates that two FcεR molecules bind one molecule of IgA (Herr et al. 2003) (see Fig. 2b). Unlike IgG or IgE, IgA glycans appear on the external side of the Cε2 domain as opposed to residing within the interstitial space of the Cε2 dimer. Herr et al., postulate that the Fcε glycan comes within eight angstroms of the FcεR, indicating a potential protein–carbohydrate or carbohydrate–carbohydrate interaction (see Fig. 2b). Structurally, FcεR resembles the Fc γamma receptors by having two extracellular Ig-like domains, a single transmembrane pass, and a short cytoplasmic region. FcεRI associates with the FcR gamma chain that contains an ITAM (immunoreceptor tyrosine-based activation motif) (Morton et al. 1995). Signaling of FcεR is dependent upon crosslinking of IgA immune complexes leading to receptor clustering and localization to lipid rafts resulting in ITAM phosphorylation causing increases in intracellular calcium levels and induction of \( \text{NADPH oxidase} \) activity in neutrophils (Lang et al. 1999). Signaling via FcεRI following antigen recognition by IgA leads to a multitude of effector responses such as ADCC, phagocytosis of bacteria and yeast, superoxide generation and release of cytokines and inflammatory mediators.

3 IgE-Mediated Immunity

When the body loses tolerance to commonly encountered foreign material hypersensitivity can result and manifest as a number of disorders including atopic dermatitis, allergic rhinitis, asthma, and food allergies. Hypersensitivity is largely driven through immunoglobulin E (IgE) and mast cells, although debate exists whether these two factors contribute to long term tissue rearrangement associated with prolonged exposure to allergens (Galli and Tsai 2012). In addition to allergic responses of the immune system IgE is also involved in anti-parasitic responses such as in defense against helminths and parasitic worms (Gounni et al. 1994). IgE is the least abundant immunoglobulin in human serum, typically found at very low levels (150–300 ng/ml) and is most likely complexed with the high affinity receptor for IgE, FcεRI on mast cells and basophils (Dorrington and Bennich 1978). Like other immunoglobulins, IgE exists as a structure consisting of two heavy and two light chains, however, it has a different domain structure and the
hinge regions are more rigid than in other immunoglobulin classes (see Fig. 3a). IgE is also heavily glycosylated with seven potential N-linked sites where carbohydrates comprise approximately 12% of the antibody mass, making it the most heavily glycosylated immunoglobulin (Arnold et al. 2004; Dorrington and Bennich 1978) (see Fig. 3a, b). Arnold et al. described the N-glycosylation of human serum IgD and IgE and identified extensive oligomannose glycosylation, including Man3, Man4, Man5, Man6, Man7, Man8, Man9, and additional hybrid structures. The total glycan pool of IgE contained approximately 14% high mannose structures, which mediate extensive interaction with MBL (Arnold et al. 2004). A significant proportion of glycans were found to be sialylated; mono-sialylated glycans accounted for 39% and di-sialylated glycans 36% of the total glycan pool. This agreed with oligomannose glycans and sialylated glycans previously reported on myeloma IgE (Dorrington and Bennich 1978; Baenziger and Kornfeld 1974a, b). Complex glycans were predominantly bi-antennary (97%) with significant amounts of core-fucosylation (68%). Bisecting N-acetylgalactosamine residues were present on approximately 15% of N-glycan structures. Site-specific glycosylation analysis of IgE has been performed recently by Plomp et al. who describe an extensive analysis of polyclonal IgE from three different sources. In all three samples of IgE isolated from pooled serum of myeloma patients, myeloma nondiseased and hyperimmune donors the Asn275 N-glycan site contained exclusively oligomannose structures (Man2–Man9). The remaining Asn21, Asn49, Asn99, Asn146 and Asn252 residues contained complex glycans which were bi-antennary core-fucosylated (98.5–100%) mono-and di-sialylated structures for the non-myeloma donors and interestingly, contained higher proportions of tri- and tetra-antennary structures and lower bi-secting GlcNAc residues in the IgE myeloma patients. The Asn264 N-glycan site was found to be unoccupied and Asn99, Asn252 and Asn275 only partially occupied (Plomp et al. 2013).

IgE N-glycans have functional importance, particularly the oligomannose carbohydrate at Asn275 which is homologous to Asn297 in IgG where the site contains a complex-type oligosaccharide. Mutation of Asn275 resulted in the loss of binding to the high affinity FcεR1, indicating the importance of this N-linked site (Nettleton and Kochan 1995). Enzymatic deglycosylation of IgE using PNGase F severely decreased the reactivity of IgE for FcεRI, suggesting that IgE glycosylation affects both structure and function of the antibody molecule (Bjorklund et al. 1999). Glycosylation also affects the binding of IgE to the low affinity FcεRII, particularly the glycan at Asn252 (Sondermann et al. 2013). However, in other reports, glycosylation of IgE has been shown to have no effect on the activity of either FcεRI or FcεRII and IgE deglycosylation was shown to have the same activity as mock deglycosylated IgE, suggesting that antibody glycosylation has only a limited affect (Vercelli et al. 1989; Basu et al. 1993). An additional nondirect function of IgE glycosylation can be found in the activity of galectins, a family of lectins that specifically bind β-galactoside. High affinity binding of galectins to the glycans of IgE regulate the activity of IgE and have an anti-allergic effect by blocking antigen complex formation (Niki et al. 2009).
3.1 FcεRI

IgE-mediated immune responses are controlled via the Fc epsilon receptor (FcεR). The high affinity FcεRI (Kₐ 10¹¹ M⁻¹) is a member of the immunoglobulin superfamily and is found on mast cells, basophils, eosinophils, and Langerhans cells where it triggers effector responses (Kraft and Kinet 2007). This can cause allergic reactions to allergens following interaction of IgE immune complexes with the FcεRI on mast cells through degranulation and release of histamine, serine proteases, proteoglycans, and inflammatory mediators. Human FcεRI exists in two forms, an αβγ2 tetramer and αγ2 trimer in which the α subunits bind IgE and the βγ
subunits signal within the effector cell. FcεRI is unique to Fc receptors; in that, it contains the additional beta chain, which acts to amplify the signal (Kraft et al. 2004). Apart from this, it has a typical interaction with the FcR gamma chain required for signaling within the effector cell. There are seven N-linked sites on FcεRI although little information exists as the occupation of these sites or the exact nature of the glycans that reside here (see Fig. 3b). It was shown by Kanellopoulos et al. and La Croix and Froese that the receptor expressed on basophilic cells contains approximately 40% of its weight due to carbohydrates, decreasing to approximately 28 kDa, following PNGase F treatment (Kanellopoulos et al. 1980; LaCroix and Froese 1993). Experiments using glycosylation inhibitors and endoglycosidase enzymes suggested that both the high affinity FcεRI and low affinity FcεRII receptors are composed of mainly complex glycans with only a single oligomannose site. Even though O-linked sites exist these experiments suggest that few O-linked glycans are present on either FcεRI or FcεRII. In the crystal structure of FcεRI carbohydrate density is indicated at three of the potential seven N-glycan sites (Asn21, Asn42, and Asn166) although there is little information as to the function of the glycans present at these sites (Garman et al. 2000). However, studies have suggested that carbohydrates on FcεRI are not required for the binding interaction with IgE and are instead needed for efficient folding and solubility of the receptor (Letourneur et al. 1995; Robertson 1993). In the crystal structure, the glycans do not extend toward the top surface of the receptor where the interaction with IgE is proposed to take place (Garman et al. 2000) (see Fig. 3b). Mutation of the N-linked sites causes misfolding and absence of N-glycosylation due to enzymatic deglycosylation affects neither the stability nor IgE binding capacity of FcεRI, again indicating that FcεRI glycans do not participate in the interaction with IgE (Letourneur et al. 1995).

FcεRI can also bind galectin-3; a known regulator of immune responses through carbohydrate interactions, resulting in crosslinking of receptor bound IgE or FcεR and activation of mast cells and basophils (Liu 2005). Galectin-3 is a lectin that specifically binds β-galactose oligosaccharides and is a low affinity IgE receptor, which can be found extracellular, cytoplasmic or nuclear where it can regulate IgE mediated immune responses with anti-allergic activities and also inflammatory mediator release (Chen et al. 2006; Frigeri et al. 1993). It has also been shown that galectin-9 specifically binds IgE via a lactose residue and prevents IgE-immune complex formation and mast cell degranulation and reduction of allergic responses (Niki et al. 2009).

### 3.2 FcεRII: The Low Affinity Fc Epsilon Receptor

FcεRII (CD23) is the low affinity IgE receptor of B-cells, macrophages and dendritic cells where it regulates the production of IgE and participates in the elimination of intracellular pathogens (Maeda et al. 1992; Vouldoukis et al. 1995). Unlike other FcRs CD23 is a C-type lectin. It is present either as a membrane bound
trimer or can be released from the cell surface by proteases such as ADAM10 to yield soluble forms (sCD23) (Dhaliwal et al. 2012). Alone, the soluble form of CD23 has a low affinity for IgE compared to FcεRI but when complexed as a soluble trimer can significantly increase the avidity and binding levels. FcεRII has a distinct role in IgE regulation due to its expression on B-cells and it is thought to contribute to both positive and negative regulation and differentiation of B-cells. Little is known about FcεRII glycosylation. There is a single N-linked glycosylation site at Asn63 and potential O-linked sites. It was shown that a 45 kDa component of CD23 was a glycoprotein containing a complex N-linked carbohydrate, several O-linked carbohydrates, and several sialic acid residues (Letellier et al. 1988). In addition, Letellier et al. show, by the use of N-glycosylation inhibitors, that the production of IgE-binding factors, derived from proteolytic cleavage of FcεRII are increased in the absence of N-glycosylation, indicating that degradation of FcεRII is inhibited in the presence of N-glycans. Sarfati et al. show that N-glycosylation controlled the activity of sCD23 and when inhibited with tunicamycin switched IgE binding factors from IgE potentiators to IgE suppressors (Sarfati et al. 1984, 1992).

4 IgG-Mediated Immunity

Immunoglobulin G (IgG) antibodies are critical glycoprotein components of the immune system which detect invading microorganisms and tumor-associated antigens and communicate this information to the innate and adaptive immune systems. The IgG antibody is the most abundant antibody isotype found in serum where it comprises approximately 75 % of total serum immunoglobulins. IgG antibodies are further divided into different subclasses (IgG1, IgG2, IgG3, IgG4) based on their abundance in serum. The subclasses differ particularly in the structure of their hinge regions. Each forms a typical Y-shaped structure composed of two heavy chains and two light chains organized into two Fab regions that bind antigen and an Fc region that is recognized by the Fc receptor specific for IgG (FcγR) (see Fig. 4a). Interaction with the FcγR on innate immune cells allows the IgG molecule to communicate with the immune system and induce immune effector functions such as ADCC, complement-dependent cytotoxicity (CDC) and phagocytosis following opsonisation of target antigens (see Fig. 4b).

4.1 The Role of N-Glycans in IgG Biology

Like other immunoglobulins, IgG is glycosylated and the addition of N-linked glycans to two conserved asparagine residues in each of the CH2 domains of the Fc region is critical for the structure and function of the antibody (Krapp et al. 2003; Arnold et al. 2007) (see Fig. 4a). In addition to glycosylation of the two canonical Asn297 residues in the Fc region, N-linked glycans are present in some 20 % of
the Fab regions in human IgG (see Fig. 4a). These glycans include bi-antennary structures that are hyper-galactosylated, fucosylated, and extensively sialylated (Mimura et al. 2007). N-linked glycans described in the Fc region of polyclonal...
IgG typically display heterogeneity and are composed of a core heptasaccharide defined by a chitobiose core (Man$_1$GlcNAc$_2$) with branching mannose residues in both $\alpha$(1–3) and $\alpha$(1–6) linkage. Further processing by the addition of GlcNAc residues in $\beta$(1–2) linkage, fucose, galactose, sialic acid, and bi-secting GlcNAc forms the mature bi-antennary IgG Fc N-glycan (see Fig. 5). In total, human serum IgG has been shown to contain up to 36 different N-glycan structures (Wormald et al. 1997). Unusual glycosylation has also been observed on IgG in the form of galactose linked $\beta$(1–4) to bisecting GlcNAc (Harvey et al. 2008). Microheterogeneity is also observed, where glycans on identical but opposing C$\beta$2 domains have been shown to vary (Jefferis et al. 1990; Masuda et al. 2000). Thus, in an otherwise symmetrical molecule, asymmetry can be introduced by differential N-linked glycosylation at individual Asn297 sites.

Glycosylation of IgG plays a number of important roles. The $\alpha$(1–6) arm of the bi-antennary glycan extends along the hydrophobic face of the C$\beta$2 amino acid backbone where the polar nature of the carbohydrate protects the underlying hydrophobic polypeptide (Lund et al. 1995). The $\alpha$(1–3) arm of the glycan extends toward the interstitial space formed by the C$\beta$2–C$\beta$3 dimer. Here, the N-linked glycans on opposite Asn297 residues interact and maintain the conformation of the Fc domain and changes in Fc glycosylation can alter the Fc conformation and affect the binding to Fc receptors (Jefferis et al. 1998; Krapp et al. 2003; Radaev and Sun 2001). Several lines of investigation have clearly indicated that changes to Fc glycosylation affect binding affinity and loss of Fc glycosylation abrogates binding altogether. Absence of glycosylation disrupts the structural integrity of the Fc region, which is required for optimal binding to the Fc receptor. In addition to maintenance of Fc structural integrity N-glycan monosaccharides play additional roles. For instance, IgG glycoforms lacking galactose (IgG G0) bind to MBL to

Fig. 5  The most common core-fucosylated bi-antennary glycan found in the Fc region of IgG. 

(a) Schematic representation of IgG bi-antennary glycan. 

(b) Cartoon representation of IgG bi-antennary glycan. Symbols and linkage positions correspond to those shown in the schematic representation. 

Human serum IgG has been found with up to 36 different N-glycan structures, predominantly in the form of core-fucosylated bi-antennary structures, as shown in a and b. Typically the N-glycan contains zero (IgG G0), one (IgG G1) or two (IgG G2) galactose residues (shown in red in the schematic representation). The glycan shown in a and b is the fully galactosylated/hypergalactosylated (IgG G2) form, however, in serum IgG1 it is the IgG G1 form which predominates. In certain conditions such as rheumatoid arthritis IgG G0 forms have been been detected in higher abundance which can effect downstream Fc$\gamma$R binding and immune function
activate complement (Malhotra et al. 1995), galactose residues are involved in placental transport of IgG and IgG galactosylation is increased in pregnant women (Simister 2003). Sialic acids have been implicated in the anti-inflammatory effects of intravenous IgG (IVIg) and desialylation abrogates this property in knockout mice (Kaneko et al. 2006). However, recently the mechanism of IVIg has been questioned and it has been shown that the interaction between DC-SIGN and IgG is not glycan dependent where Yu et al. challenge the idea that DC-SIGN directly binds to IVIG, suggesting that DC-SIGN is unlikely to be the receptor. (Yu et al. 2013).

To fucosylate or not to fucosylate?
Core-fucosylation of IgG has been the subject of intensive research ever since afucosylated IgG was shown to exhibit improved binding to activating Fc gamma receptor and enhanced ADCC (Shields et al. 2002; Okazaki et al. 2004; Mori et al. 2004; Yamane-Ohnuki et al. 2004; Kanda et al. 2007; Natsume et al. 2005; Iida et al. 2006; Satoh et al. 2006; Ferrara et al. 2006a, b). With such a significant impact on immune effector function, the biopharmaceutical industry has vigorously pursued the generation of afucosylated monoclonal antibody therapeutics. Genetic elimination of fucosyltransferase 8 (FUT 8) in Chinese hamster ovary cells has been a successful approach for the prevention of core fucosylated IgG (Yamane-Ohnuki et al. 2004). Alternative methods such as the overexpression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) as a means of eliminating the substrate for fucosyltransferase and modification of enzymes involved in the N-linked glycan biosynthetic pathway have also been developed with significant success (Umana et al. 1999; von Horsten et al. 2010; Zhou et al. 2008). The majority of approved mAb therapeutics that target ADCC have been engineered for use in anti-cancer therapies where the mAb targets a cell surface receptor associated with a particular tumor. In this situation, the absence of core α(1–6)-linked fucose has been associated with improved cytolytic activity and efficacy of the mAb (Shields et al. 2002; Nimmerjahn and Ravetch 2005). This has been most commonly studied in B-cell lymphomas where the monoclonal antibody (rituximab) recognizes the cell surface receptor CD20 on B-cells and induces natural killer (NK) mediated ADCC. Another very successful anti-tumorigenic mAb is herceptin, which targets the Her2 receptor expressed on some breast cancer tumors. Due to the remarkable success of this approach, industry has moved toward generation of cell lines with genetic modifications that directly influence core fucosylation.

4.2 Role of Glycosylation in FcγR Biology

IgG antibodies communicate with the immune system via interaction with the plasma membrane bound Fcγ receptors found on innate immune cells. FcγRs are typically single pass transmembrane glycoproteins belonging to the immunoglobulin-like superfamily (IgSF), defined by characteristic domains based on two
sheets of antiparallel β-strands (Williams and Barclay 1988) (see Fig. 6). The FcγR family is broadly categorized into three groups: FcγRI, FcγRII, and FcγRIII. Variability is observed within each group at the genomic, transcriptomic, and proteomic level where multiple genes, transcripts, and polymorphic variants all contribute to FcγR complexity. Functionally, the individual FcγR groups coordinate distinct functions. The FcγRI family are characterized by their high affinity ($10^9$ M$^{-1}$) for IgG while the remaining families are low affinity ($10^6$ M$^{-1}$) receptors. Extensive variability is also observed in the cytoplasmic domain of each receptor, where signaling can be either through association with the γ-chain dimer (for FcγRI and FcγRIII) or through integrated signaling motifs (for FcγRII). Signaling occurs through either an immunotyrosine-like activation motif (ITAM) for activating FcγRI, FcγRIla, FcγRIII or an immunotyrosine-like inhibitory motif (ITIM) for inhibitory FcγRIIib.

Emerging from the shadows of IgG and Fc glycosylation is the growing complexity of Fcγ receptor biology and the role of these receptors in health and disease. While intensive efforts have been directed towards manipulating IgG glycosylation for enhanced biological activity, Fcγ receptors have traditionally been used in a limited role to investigate monoclonal antibody safety and efficacy. However, this is no longer the case and the complexity of Fcγ receptor biology and the role of glycosylation is becoming increasingly evident. Research into the glycosylation of Fcγ receptors was initiated over three decades ago, however it is only now that are we beginning to appreciate the precise function of the carbohydrate moieties in relation to IgG binding and associated biological activity. Surprisingly, due to the
difficulty in obtaining sufficient amounts of material for analysis very little is known about the glycosylation of Fcγ receptors as they occur in their natural environments, bound to the cell surface membranes of lymphocytes such as neutrophils, monocytes, macrophages, B-cells and NK cells. Each Fcγ receptor contains at least two N-linked glycosylation sites with up to seven potential sites observed in the high affinity FcγRIa. The vast majority of glycan data exists for FcγRIIIa. We now have structural information which shows how human FcγRIIIa associated sugars are involved in carbohydrate–carbohydrate interactions with the Asn297-linked sugars of afucosylated human IgG1 (Ferrara et al. 2011). Glycosylation data have also been reported for recombinant soluble human FcγRIIa and FcγRIIIa (Takahashi et al. 1998, 2002) and recently glycan data have been described for the family of recombinant receptors: FcγRIa, FcγRIIa, FcγRIIib, FcγRIIIa, and FcγRIIIb expressed in the murine cell line NS0 (Cosgrave et al. 2013). Interestingly, aglycosylated Fcγ receptors expressed in E. coli and purified from inclusion bodies still appear to retain the ability to bind IgG (Sondermann and Jacob 1999; Sondermann et al. 2000; Maenaka et al. 2001) which brings into question the exact nature of Fcγ receptor glycosylation in IgG binding.

In the light of the emerging importance of Fcγ receptor glycosylation in IgG function there is a critical need for detailed knowledge of the glycosylation state of natural FcγRs of immune cells. One likely possibility is that an Fcγ receptor demonstrates differential glycosylation depending on the cell type. Ample evidence of this was provided by Edberg et al. (1990); (Edberg and Kimberly 1997). Knowledge that Fcγ receptor glycosylation changes depending on the immune cell will help to understand the associated immunological outcomes. Following cytokine-mediated immune activation, innate effector cells are known to up-regulate Fcγ receptors as has been shown with IFN-γ and monocytes (Fleit and Kobasiuk 1991; Fairchild et al. 1996). In this case, changes in Fcγ receptor glycosylation due to immune cell activation may shed light on glycoform preferences for improved IgG binding. Resting or inactive innate effector cells may glycosylate Fcγ receptors in a manner that promotes dissociation as a mechanism to avoid inadvertent activation. Upon stimulation with appropriate mediators, Fcγ receptor glycosylation may change to recruit and retain circulating antibodies. Rapid upregulation of Fcγ receptor expression is likely to have an impact on glycosylation. The question is does this “activated” Fcγ receptor glycoform have an improved or dampened interaction with IgG or does a change in FcγR glycosylation disrupt or modify cell surface interactions? It is therefore of paramount importance to characterize natural Fcγ receptor glycosylation. Of equal importance is to learn how immune responses alter Fcγ receptor glycosylation. Immune activation may induce the expression of Fcγ receptors with improved binding, possibly through altered glycosylation. The glycosylation may also promote engagement of other molecules that work to extend the presence of receptors on the cell surface. An example of this could be the role of galectin in cellular distribution and trafficking of the epidermal growth factor receptor (EGFR) (Merlin et al. 2011; Liu et al. 2012). During an immune response, the preferable scenario for the innate effector cell is to express the Fcγ receptor on the cell surface to scavenge for IgGs. In fact, it is
most likely that glycosylation changes to promote cross-linking of antibodies. If binding improves and dissociation is reduced, then Fcγ receptors will become occupied by antibodies which may not be involved with a specific immune response. Perhaps more likely is a situation in which clustering of the Fcγ receptors is improved as this will more readily lead to immune cell activation. Another possibility is that glycosylation of inhibitory FcγRs may change to reduce interaction with IgGs. This would effectively eliminate inhibitory Fcγ receptors from cluster points and in the process alter the A/I ratio and induce cell activation. Alternatively, situations of immune tolerance may see glycosylation of inhibitory FcγRIIb change to promote binding and retention/clustering of autoantibodies.

4.3 FcγRI (CD64)

The FcγRI family is the high affinity Fcγ receptor and is structurally distinct from FcγRII and FcγRIII by the presence of a third extracellular D3 domain (see Fig. 6). This additional domain has been shown to account for the high affinity property of the FcγRI family (Harrison and Allen 1998; Allen and Seed 1989; Lu et al. 2011). FcγRIIa is primarily expressed by monocytes and macrophages although myeloid cell lines induced with cytokines such as IFN-γ are capable of FcγRI expression (Fairchild et al. 1996). The FcγRI family is the only Fcγ receptor class capable of appreciably binding monomeric IgG in vivo (Bruhns et al. 2009). Similar to the FcγRIII family, FcγRI requires the interaction with the γ-chain homodimer for cell surface expression and signal transduction (van Vugt et al. 1996; Ernst et al. 1993).

FcγRIa is a 374 amino acid protein with seven potential N-glycosylation sites. In the absence of post-translational modifications, FcγRI is a 42.6 kDa single pass transmembrane protein with short cytoplasmic region. At present, little is known about the site occupancy of these N-glycosylation sites or the carbohydrates that reside there. A study of neutrophil FcγRIa did, however, reveal that the receptor is heavily glycosylated with approximately 30 % of its weight due to carbohydrates. There are a larger number of glycosylation sites associated with this receptor in comparison to other members of the FcγR family, largely due to the extra D3 domain, which contains two putative N-linked sites. The crystal structure of soluble extracellular FcγRIa observed and modeled N-glycans at six asparagine residues (Asn59, Asn78, Asn152, Asn159, Asn163, Asn195) but little information as to the exact nature of the carbohydrates exists (Lu et al. 2011). As this is the high affinity receptor for IgG it is likely that the glycans that reside on the N-glycosylation sites in this extra domain will play a role in the interaction with IgG. Further studies are needed to investigate this assumption. Recombinant FcγRIa expressed in murine cells was heavily glycosylated with multiantennary structures, core and outer arm fucosylation with predominantly neutral and mono-sialylated glycans and smaller amounts of di- and tri-sialylated structures. In addition, large amounts of the immunogenic carbohydrates gal-α(1,3)-gal(α-gal) and N-glycoly neuraminic acid (NGNA) were discovered (Cosgrave et al. 2013).
4.4 FcγRII (CD32)

The FcγRII family of Fc receptors are structurally and functionally distinct from FcγRI and FcγRIII primarily due to an integrated signaling motif located in the C-terminal cytoplasmic region of the protein. FcγRII is categorized into FcγRIIa, FcγRIIb, and FcγRIIc, where six transcripts in total have been described (Warmerdam et al. 1993; Qiu et al. 1990). Two transcripts arise from the FcγRIIa gene (FcγRIIa1 and FcγRIIa2), where FcγRIIa2 is believed to be a soluble form of FcγRIIa. Three separate transcripts arise from FcγRIIb (FcγRIIb1, FcγRIIb2, and FcγRIIb3) and only one arises from FcγRIIc. With the exception of FcγRIIa2, all transcripts give rise to single pass transmembrane glycoproteins. Due to the significant differences in biological function between FcγRIIa and FcγRIIb, each will be discussed separately.

4.4.1 FcγRIIa

FcγRIIa is a 40 kDa single pass transmembrane glycoprotein capable of potent inflammatory response activation. The FcγRIIa family is comprised of FcγRIIa1 and FcγRIIa2, where the latter is believed to represent a soluble form of the receptor (van den Herik-Oudijk et al. 1994). Structurally, FcγRIIa demonstrates significant homology to FcγRIIb in the extracellular domain with approximately 92% amino acid identity, however, significant differences are present in both the transmembrane and cytoplasmic domains where FcγRIIa contains an activatory ITAM motif and FcγRIIb has an inhibitory ITIM motif. FcγRIIa is the most widely expressed FcγR, found on neutrophils, eosinophils, B lymphocytes, platelets (Rosenfeld et al. 1985), mast cells (Sylvestre and Ravetch 1996), Langerhans cells (Schmitt et al. 1990), placental endothelial cells (Sedmak et al. 1991), and dendritic cells (Sallusto and Lanzavecchia 1994).

The low affinity FcγRIIa is a 317 amino acid protein with two N-linked glycosylation sites. Glycosylation of this receptor expressed in insect cells was reported to be uncharged suggesting no sialylation of N-glycans (Sondermann et al. 1999). Glycosylation was also reported to have no effect on binding of IgG. Powell et al. reported that expression of FcγRIIa in CHO cells was found to display micro-heterogeneity resulting from extensive sialylation on both N-linked sites while the same receptor derived from baculovirus infected insect cells contained only simple Man3 glycan structures (Powell et al. 1999). FcγRIIa expressed in NS0 cells contained complex glycosylation with multiantennary structures, extensive core and outer-arm fucosylation and immunogenic x-gal and NGNA, similar to FcγRIa (Cosgrave et al. 2013). However, little is known as to the site-occupancy of the N-linked sites or the function of FcγRIIa glycosylation. In addition, there is no information currently available relating to natural FcγRIIa glycosylation.
4.4.2 FcγRIIb

FcγRIIb is a 34 kDa (peptide mass) single pass membrane glycoprotein and is the most widely expressed FcγR. In contrast to other FcγRs, FcγRIIb contains an inhibitory signaling motif (ITIM) in its cytoplasmic tail. FcγRIIb has a rare polymorphism resulting from an SNP, causing an amino acid change at position 232 to an isoleucine (Li et al. 2003). The functional consequence of this variation is the failure of FcγRIIb to associate in lipid rafts, thereby losing the ability to dampen immune cell activation through activatory Fc receptors. This has been shown in SLE (Floto et al. 2005; Kono et al. 2005). Two isoforms of FcγRIIb, referred to as FcγRIIb-1 and FcγRIIb-2, exist as a result of alternative splicing and are expressed differentially depending on the cell type, FcγRIIb-1 is exclusively expressed on B-cells and FcγRIIb-2 is expressed on all other FcγR cell types, except NK cells (Nimmerjahn and Ravetch 2008). FcγRIIb is a 310 amino acid protein containing three potential N-glycosylation sites. Similar to other FcγRs little information exists as to the site occupancy or nature of glycosylation at these sites. To our knowledge, the only FcγRIIb glycan data come from a recombinant form expressed in murine cells. In common with other FcγRs expressed from this source FcγRIIb contained multiantennary structures, limited sialylation and extensive immunogenic carbohydrate epitopes (Cosgrave et al. 2013). No information is currently available relating to natural FcγRIIb glycosylation.

4.4.3 FcγRIIc

FcγRIIc is believed to have occurred from a unequal genetic cross-over event that effectively brought the 5′ region of FcγRIIb to the 3′ region of FcγRIIa (Warmerdam et al. 1993). Interestingly, functional FcγRIIc has been identified on NK cells, where four separate mRNA transcripts were isolated from NK cells (Metes et al. 1998). Four distinct isoforms of the protein exist with molecular weights ranging from 25.9 to 35.5 kDa and 234–323 amino acids. There are also three potential N-linked sites although no information exists as to the glycosylation of this protein.

4.5 FcγRIII (CD16)

4.5.1 FcγRIIIa

The biology of the FcγRIII family has been the subject of intensive focus, largely due to the role of activating FcγRIIIa in NK cell activity and ADCC. FcγRIIIa demonstrates unique structural characteristics by requiring association with the γ-chain dimer (similar to FcγRI) and further association with the ζ-chain when expressed by NK cells, where it has been demonstrated that FcγRIII expression requires association with an accessory chain (Kinet 1992; Hibbs et al. 1989).
FcγRIIIa expresses two immunoglobulin-like C2-type domains (similar to FcγRII). Perhaps most interesting is the diversity within the two FcγRIII family members: FcγRIIIa and FcγRIIIb. The extracellular domains of both receptors are nearly homologous with the exception of six amino acids. Interestingly, a key residue at position 203 influences whether FcγRIII becomes a single pass membrane bound receptor (Phe203) or membrane-associated through a GPI-anchor (Ser203). FcγRIIIa is widely distributed across the hematopoietic system, found on cells such as monocytes and NK cells but has different properties depending on the cell such as altered sensitivity to trypsin (Perussia and Ravetch 1991). There is emerging evidence that FcγRIIIa is expressed by γδT cells and contributes to disease progression of multiple sclerosis by ADCC dependent means (Chen and Freedman 2008), although FcγR expression in any form by T-lymphocytes is a widely debated subject.

Most of our knowledge of FcγR glycobiology comes from the low affinity activating FcγRIIIa of monocytes and NK cells. FcγRIIIa is a 254 amino acid protein with five potential glycosylation sites. Early seminal work on FcγRIIIa glycosylation was performed by Edberg et al. who showed that cell type specific glycoforms of the receptor existed that exhibited differential ligand binding (Edberg et al. 1989, 1990). Using lectin-binding experiments FcγRIIIa from NK cells was shown to display N-glycans of predominantly oligomannose type while the same receptor expressed by monocytes displayed complex type oligosaccharides. Furthermore, removal of high mannose oligosaccharides from FcγRIIIa resulted in a change in the binding of aggregated IgG (Kimberly et al. 1989). Edberg et al. also showed that the receptor expressed by monocytes was distinct from that displayed by NK cells (Edberg and Kimberly 1997) and that FcγRIIIa on NK cells has the ability to bind monomeric IgG whereas FcγRIIIa on monocytes lacks this ability. Interestingly, at physiological IgG concentrations, FcγRIIIa is saturated but can more easily be displaced by competing molecules (Mab3G8) than the NK cell equivalent. This data suggests that monocytes utilize FcγRIIIa differently than NK cells and it is convincing to attribute this difference in FcγRIIIa and IgG binding to glycosylation. Interestingly, these discoveries suggest that differential glycosylation potentially plays a role in influencing Fc-FcγR affinity, and therefore immune cell activation.

More recently, a huge amount of proteomic and glycomic information has been described for recombinant forms of FcγRIIIa and importantly the N-linked glycan site occupancy of the receptor. Two N-linked sites of FcγRIIIa directly regulate the binding of IgG. Mutagenesis of the N-linked glycan at Asn162 significantly reduced the binding of IgG1, demonstrating a dependence of this interaction on FcγRIIIa glycosylation at this particular site (Ferrara et al. 2006b) (see Fig. 4b). In addition, the N-linked glycan at position Asn45 was shown to have an inhibitory role in IgG binding, where removal of the N-linked site at this position dramatically improved the binding of IgG1 (Shibata-Koyama et al. 2009). It has been proposed that the glycan at position 45 within the D1 domain of FcγRIIIa acts to stabilize the D2 domain and protect it from intracellular proteolytic degradation. As a consequence, the Asn45 occupied site exhibits a reduced binding affinity of afucosylated IgG1 to FcγRIIIa. A site-specific analysis of recombinant FcγRIIIa
expressed in HEK293 and CHO cells was performed by Zeck et al., who showed that specific types of glycans were found on particular N-linked sites including multi-antennary structures, sialylation, and core-fucosylation. Significant characteristics of the expression host were reported, such as LacdiNAc (HexNAc–HexNAc) structures from HEK293 cells, and the authors also report on the effect on IgG binding of larger HEK293 glycans compared to smaller CHO glycans located on the same sites (Zeck et al. 2011). Although these are non-natural sources it does provide evidence for site-specific glycans, which may mediate the IgG interaction. Recently, it was shown at the molecular level that a unique carbohydrate–carbohydrate interface was involved in the interaction between afucosylated IgG1 and FcγRIIIa, which explained the increased affinity for afucosylated antibody through lack of steric hindrance, which is present for core-fucosylated forms (Ferrara et al. 2011) (see Fig. 4b). Glycosylation of recombinant FcγRIIIa from NS0 cells has also been described (Cosgrave et al. 2013).

4.5.2 FcγRIIIb

FcγRIIIb is a GPI-anchored protein present on macrophages and neutrophils and has no currently known cytosolic signaling domain. FcγRIIIb activation and crosslinking on neutrophils have been shown to cause neutrophil degranulation and generation of reactive oxygen intermediates, which can in turn increase FcγRIIIa activation by increasing avidity and efficiency (Salmon et al. 1995). These cooperative FcγR effects show the potential for synergistic FcγR activation. In addition, FcγRIIIb has been shown to associate with complement receptor 3 (CR3) in fibroblast transfectants, highlighting the potential for inter-receptor interactions and activation (Poo et al. 1995).

More information is available for FcγRIIIb glycosylation than for the other FcγRs, with the exception of FcγRIIIa. FcγRIIIb expressed on neutrophils is a 233 amino acid protein with six potential N-linked sites that contain oligomannose glycans that influence the properties of the IgG-receptor binding interactions (Kimberly et al. 1989). The apparent molecular weight of FcγRIIIb varies from 50 kDa to 80 kDa due to differential glycosylation (Ravetch and Perussia 1989; Scallon et al. 1989; Edberg et al. 1989; Huizinga et al. 1990). Furthermore, the NA polymorphic variations (NA1 and NA2) of FcγRIIIb are also believed to demonstrate heterogeneity in glycosylation (Kimberly et al. 1989). Galon et al. (1997) expressed soluble human FcγRIIIb in both E. coli and baby hamster kidney (BHK) cells and observed a decrease in binding affinity due to the presence of N-linked glycans (Galon et al. 1997). However, N-linked carbohydrates were not determined. N-linked glycans were reported for FcγRIIIb from BHK cells by Takahashi et al., who described multi-antennary structures containing up to four GlcNAc residues and minimal sialic acid capping (Takahashi et al. 2002). Glycosylation of recombinant FcγRIIIb from murine cells was also described and similar to BHK cells consisted of multiantennary structures and incomplete sialic acid capping and similar to other receptors expressed in murine cells with extensive immunogenic ζ-
gal and N-glycolylneuraminic acid carbohydrate epitopes (Cosgrave et al. 2013). Site-specific and natural glycosylation information is currently not available for FcγRIIIb.

5 FcRn: The Neonatal Fc Receptor

The neonatal Fc receptor (FcRn) is unlike the other Fc receptors and is more similar in structure to MHC class I. The mature FcRn receptor consists of a complex of two subunits: p51 and p14 (β2-microglobulin) and forms an MHC class-I-like heterodimer (Simister and Mostov 1989; Burmeister et al. 1994a, b). The receptor is located in numerous tissues and organs, such as the vascular endothelium and myeloid derived antigen presenting cells (APC), such as monocytes, macrophages, and dendritic cells where it functions in the transfer of IgG from mother to fetus (Simister 2003; Simister and Mostov 1989). This process is critical for transferring humeral immunity from mother to child. The receptor is found in the placenta to help facilitate this critical process. FcRn is also involved in recycling of IgG and regulates its serum half-life, maintaining its serum concentration and regulating IgG homeostasis (Ward et al. 2003; Antohe et al. 2001; Roopenian and Akilesh 2007). Only at acidic pH, such as in endocytic vacuoles will FcRn bind IgG, releasing it at physiological pH. By recycling IgG from acidic endosomes and releasing it back at the cell surface FcRn increases the half-life of IgG, which is needed at high serum concentration to fight infection. FcRn can also bind IgG immune complexes resulting in transport to lysosomes in dendritic cells for antigen presentation (Qiao et al. 2008; Yoshida et al. 2004).

FcRn contains a single N-linked glycosylation site in the α2 domain in the 365 amino acid large subunit (p51). Human and rat FcRn differ by the number of N-glycan sites, rat FcRn has four sites in the α1, α2, α3 domains, whereas the human receptor has a single glycosylation site (Asn125). The crystal structure of rat FcRn revealed that the carbohydrate on Asn128 is at the interaction site for IgG and is possibly involved in the IgG interaction (Burmeister et al. 1994a). The glycan moiety makes contact with the Fc region of the IgG molecule and has been postulated to help stabilize the complex formation through a carbohydrate interaction with IgG (Vaughn and Bjorkman 1998). Recently, information became available on the glycosylation of human and rat FcRn expressed in canine MDCK II cells which showed that the receptor from both sources contained both oligomannose and complex glycans and that following EndoH removal of high manose structures the size of the glycoprotein was reduced from 52 to 37 kDa (Kuo et al. 2009). However, the exact monosaccharide compositions were not reported. Kuo et al. report increased surface expression of FcRn following the introduction of additional N-glycan sites to the protein and show that carbohydrates are involved in the direction of IgG transport by FcRn and that direction of transport mediated by human FcRn is reversed by the addition of N-glycan sites, making it more like the rodent form.
6 Closing Remarks

Immunoglobulins and Fc receptors are complex glycoproteins and key components of both the innate and adaptive immune systems. Glycosylation of immunoglobulins (IgA, IgD, IgE, IgG) has been well studied and the sugars attached to the conserved asparagine residues in the Fc region are undeniably critical to the antibodies function and its communication with the immune system. Exciting discoveries that show individual monosaccharide residues of Fc N-glycans modulate binding of antibodies to immunoglobulin receptors on immune effector cells and drive particular immune responses is intriguing and has led to an explosion of research into the influence of glycosylation in antibody mediated responses. Corefucosylation and its influence on activating FcγRs and antibody mediated anti-tumor activities have led to huge industrial interest into antibody glycosylation. Molecular mechanisms at atomic level for such interactions are now being elucidated. Therapeutic antibodies have had remarkable success in the treatment of many diseases, from cancer to autoimmune disorders and glycosylation is integral and crucial to past and future successes. Emerging now from the shadows of antibody glycosylation are the Fc receptors. It is true to say that we do not yet fully understand the role of these critical receptors in health and disease, particularly the role of glycosylation. Glycosylation of Fc receptors is far more complex than for antibodies and we have very little information as to how these receptors are glycosylated in their natural environment, by cells such as macrophages, B-cells and NK cells. Even though significant information on how these receptors are glycosylated was revealed more than 30 years ago it is fair to say that we are just beginning to understand the true importance of receptor glycosylation in the interaction with antibody and downstream immunological response. It is intriguing to consider the role that sugars could play when a particular immune cell encounters antibody immune complexes and the potential control mechanisms mediated by carbohydrates. It remains a challenge for the future to gain a more complete understanding of the glycosylation of antibody Fc receptors in both activated and dormant states and in both healthy and disease situations. Fc receptors for IgA at mucosal surfaces, for IgE in allergic reactions and defense against parasites, IgG in infection and anti-tumor activities and other antibody-mediated responses is becoming more and more critical to successful future clinical therapies and successes.

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