Glycoconj J (2013) 30:735–745
DOI 10.1007/s10719-013-9481-y

IgG-Fc *N*-glycosylation at Asn297 and IgA *O*-glycosylation in the hinge region in health and disease

Jing Xue · Li-Ping Zhu · Qiang Wei

Received: 1 April 2013 / Revised: 16 May 2013 / Accepted: 16 May 2013 / Published online: 20 June 2013
© Springer Science+Business Media New York 2013

Abstract Immunoglobulins (Igs) are the major molecules secreted by B lymphocytes during an adaptive immune response. They are glycoproteins with distinctive glycosylation patterns, resulting in wide variations in the number, type and location of their oligosaccharides in each isotype and subclass. The sugars play specific structural roles, maintaining and modulating effector functions of Igs. Aberrant glycosylation might contribute to disease pathogenesis. This review will focus on the glycosylation of IgG and IgA because they have been studied more extensively than other immunoglobulins. Rheumatoid arthritis and IgA nephritis are used to describe the association of glycosylation aberration and disease pathogenesis.

Keywords Immunoglobulin · Glycosylation · Health · Disease

Abbreviation

Ig	Immunoglobulin
Fab	Fragment of antigen binding
Fc	Crystallizable fragment
GlcNAc	*N*-acetylglucosamine
Fuc	Fucose
Man	Mannose
Gal	Galactose
NeuAc	*N*-acetylneuraminic acid
GalNAc	*N*-acetylgalacosamine
MBL	Mannose-binding lectin
ADCC	Antibody-dependent cell-mediated cytotoxicity
CDC	Complememt-dependent cytotoxicity
CpG	Demethylated deoxycytidyl-deoxyguanosine
CpG-ODN	Oligodeoxycynucleotides

J. Xue · Q. Wei (✉)
Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (CAMS) and Comparative Medicine Center, Peking Union Medical College (PUMC), Key Laboratory of Human Disease Comparative Medicine, No 5 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China
e-mail: weiqiang@cnilas.pumc.edu.cn

L.-P. Zhu (✉)
Department of Immunology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (CAMS) and School of Basic Medicine, Peking Union Medical College (PUMC), No 5 Dong Dan San Tiao, Beijing 100005, China
e-mail: zhulp@pumc.edu.cn

Introduction

Glycosylation, an enzymatic process catalyzed by various glycosyltransferases and glycosidases, is an important post-translational protein modification that links saccharides, proteins and lipids. Protein glycosylation produces monomeric or multimeric glycan linkages to proteins that are essential for cell viability and normal functions. It has been demonstrated that changes in glycans significantly modulate the structure and function of glycoprotein polypeptides [1]. This consequently affects the biological behavior of cells, including adhesion, molecule trafficking and clearance, receptor activation, signal transduction, endocytosis, and the interaction between a cell and its environment [2, 3]. Deregulation of glycosylation is associated with a wide range of diseases, including cancer, diabetes, cardiovascular, congenital, immunological and infectious disorders [1, 2].

Mammalian immunity is profoundly affected by protein glycosylation. Marth and Crewel reviewed the current understanding of the role of mammalian glycosylation in immunity [4]. Glycosylation has been associated with many immune activities, including immune cell differentiation and maturation, antigen and molecule recognition, signaling and cell activation, cell adhesion, expression and secretion of immune molecules, immune tolerance, and apoptosis.

Immunoglobulins (Igs) are the major molecules secreted by B lymphocytes during an adaptive immune response. Arnold *et al.* characterized the distinctive glycosylation of each Ig isotype and subclass and showed a wide variation in the number, type, and location of their oligosaccharides [5]. Disease-associated alterations of Ig glycosylation were referred in the review, which provided diagnostic biomarkers or contributed to disease pathogenesis. Protein oligosaccharides also provide important recognition epitopes that bind lectins, endowing the immunoglobulins with an expanded functional repertoire. This review will focus on the glycosylation of IgG and IgA because they have been studied more extensively than other immunoglobulins. Rheumatoid arthritis and IgA nephritis are used as examples of glycosylation aberrations associated with disease pathogenesis.

Ig Glycosylation

IgG glycosylation

Both Fab and Fc portions of all IgG subclasses are *N*-glycosylated. The Fab glycosylation is variable. However, the Fc portion has a single *N*-linked glycosylation site at amino acid 297 of the heavy chain. The *N*-glycan at Asn297 has a predominantly biantennary complex structure with a tri-mannose core of $GlcNAc_2$-Man_3-$GlcNAc_2$. The addition of a fucose to the reducing terminal GlcNAc, the addition of a bisecting GlcNAc, GlcNAcβ1-2, Galβ1-4GlcNAcβ1-2, NeuAcα2-6Galβ1-4GlcNAcβ1-2 to tri-mannose core results in significant composition heterogeneity of the glycan. Sialylated *N*-glycans are actually very minor and non-sialylated neutral *N*-glycans are abundant in the normal condition. Thus, Asn^{297} *N*-glycans may contain 0, 1, or 2 terminal Gal residues (G0, G1, or G2) and 1 or 2 terminal NeuAc residues (S1 or S2) in their antennae (Fig 1). However, the major structure is non-bisected fucosylated bi-antennary glycan (~80 %) at one side and bisected bi-antennary glycan is between 10 % and 20 % at the other side [6, 7].

Asn297 *N*-glycan heterogeneity exists in various physiological conditions. For example, human IgG glycosylation was reported to change with age [8, 9]. The incidence of non-galactosylation in IgG reached ~30 % in the first year in newborns, decreased to ~20 % approximately 25 years of age, then continuously decreased with aging, but increased again to reach ~30 % around age 70. Renee Ruhaak *et al.* also observed decreased galactosylation of IgG with aging [10]. In addition, they found that the incidence of bisecting GlcNAc for galactosylated glycoforms increased with age. Interestingly, decreased levels of non-galactosylated glycoforms containing bisecting GlcNAc might be an early feature of longevity in people below, but not above, 60. In addition, an association was reported between sex and IgG galactosylation [10]. Rook *et al.* reported the effect of pregnancy on IgG glycosylation [11]. They found that the incidence of IgG-associated agalactosyl *N*-linked oligosaccharides decreased during normal human pregnancy and rose to values higher than before conception. The mechanism of Asn297 *N*-glycan heterogeneity has not yet been clarified. It is known that oligosaccharyltransferases play important roles in the catalysis of protein *N*-glycosylation. Kelleher *et al.* found that oligosaccharyltransferase isoforms containing different catalytic STT3 subunits exhibited distinct enzymatic properties in mammalian cells [12]. Prados *et al.* recently examined effect of progesterone on IgG Fab *N*-glycosylation and observed that progesterone induced a switch to STT3-B/STT3-A expression, which consequently modulated the Fab *N*-glycosylation [13].

IgA glycosylation

IgA is the most glycosylated form of Ig. Both subclasses carry several *N*-linked carbohydrates with a glycan at amino acid 263 in the C_H2 domain and a glycan at amino acid 459 in the C_H3 domain. IgA2 has two additional *N*-linked glycans at amino acid 166 in the C_H1 domain and at amino acid 337 in the C_H2 domain. *N*-linked glycans contribute to 6–7 % of the molecular mass of IgA1 and 8–10 % of IgA2 [14].

The hinge region of IgA1 comprises 18 amino acids (VPSTPPTPSPSTPPTPSPS), which are rich in proline, serine and threonine. The last two residues are sites for the attachment of up to five *O*-linked glycan chains consisting of GalNAc [15]. IgA2 lacks a 13-amino acid sequence in the hinge region. The IgA1 *O*-glycans are of the core-1 type. *O*-glycan chains are formed in the Golgi apparatus during IgA1 synthesis by the sequential action of glycosyltransferases. The initial linking of GalNAc to the protein backbone to form Tn antigen is catalyzed by one member of the UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetyl galactosaminyltransferase family, known as GalNAc-T2 [16]. Gal may be linked to the GalNAc by a core-1 β1,3-galactosyltransferase, known as C1GalT1, to form the disaccharide Gal-β1-3-GalNAc (T antigen) [17]. C1GalT1 activity requires co-expression of the chaperone protein core-1 β3-Gal-T-specific molecular chaperone (COSMC), which may modulate galactosaminyltransferase function [18]. The core-1 may be extended with one or two sialic acid units that are added during the catalysis of Gal:α2,3-sialyltarnsferase (ST3 Gal) or GalNAc:α2,6-sialyltransferase (ST6 GalNAc2) to form sialyl-Tn, monosialyl-T or disialyl-T [19]. Fig 2 shows a diagram of normal and aberrant *O*-glycosylation with galactose-deficiency of IgA1. The most common forms include core-1 and its mono-and di-sialylated forms. Gal-deficient variants with terminal GalNAc or sialylated GalNAc exist, but they represent a minor percentage of the *O*-glycans in the serum IgA1 (Fig 2) [20]. SIgA, a J chain-linked IgA, is synthesized and secreted by plasma cells in the

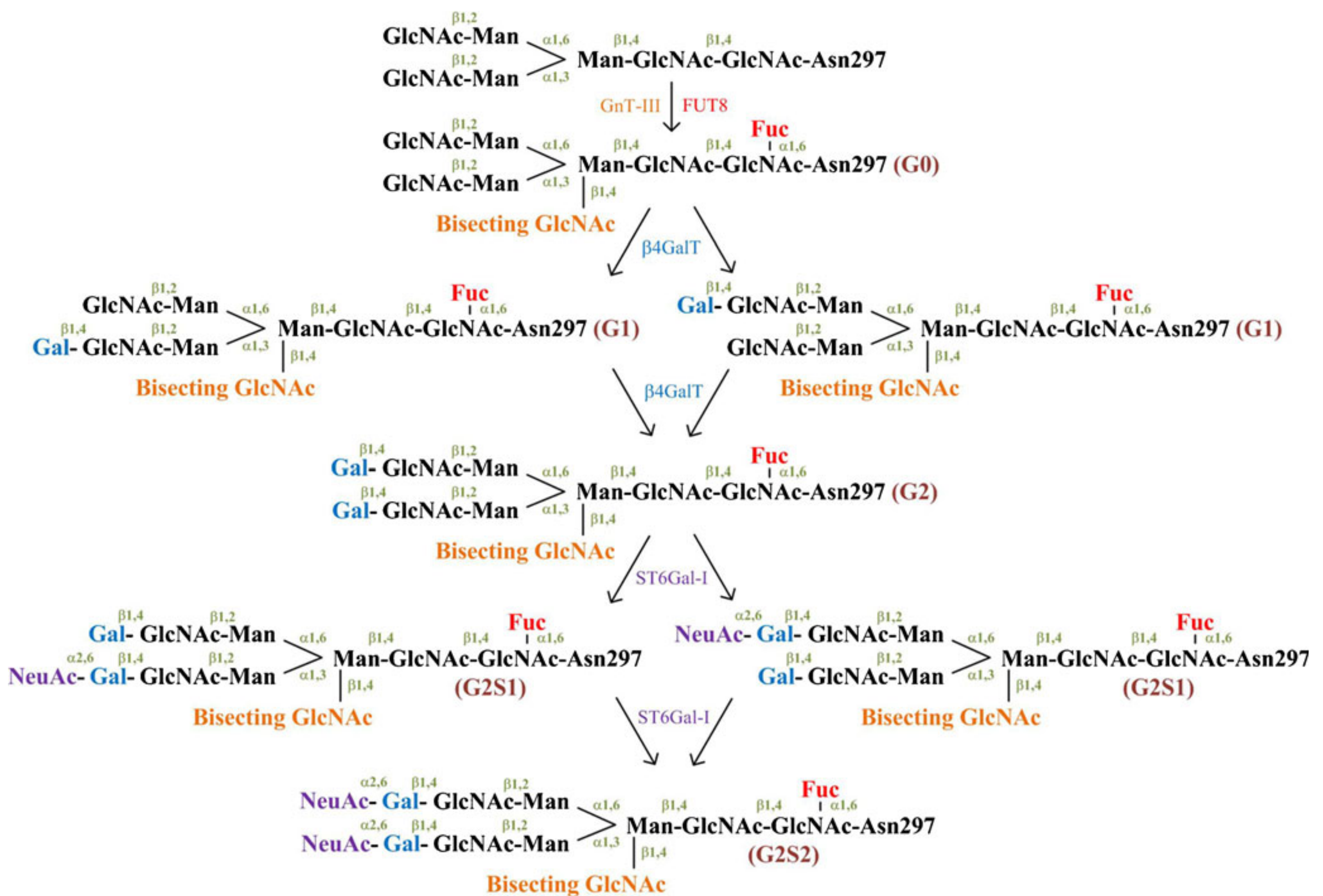

Fig. 1 Asn297-*N*-glycosylation and heterogeneity of Asn297-*N* glycan in IgG. Asn297-*N*-glycan is a complex saccharide with biantennary strucure. It has a tri-mannose core of $GlcNAc_2$-Man_3-$GlcNAc_2$. Heterogeneity of the glycan is resulted from the addition of fucose to the reducing terminal GlcNAc by α6-fucosyltransfurase (FUT8), the addition of a bisecting GlcNAc by *N*-acetylglucosaminyltransferase (GnT-III), the addition of Gal to GalNAc-Man by β4-galactosyltransferase (β4galT) and the addition of NeuAc to Gal-GalNAc-Man by Gal: α2,6-sialyltransferase (ST6Gal-I) to tri-mannose core. Asn297 *N*-glycan may contain 0, 1, or 2 terminal Gal residues (G0, G1, or G2) and 1 or 2 terminal NeuAc residues (S1 or S2) in their antennae

lamina propria and transported to the laminal surface through transcytosis. It is then released into the mucosal cavity *via* mucosal endothelial cells. SIgA contains a secretory component with seven *N*-glycosylation sites and a J chain with one *N*-glycan. SIgA is normally IgA2 [14].

Glycosylation of other Ig isotypes

The Mu chain has five *N*-glycosylation sites. Glycoforms of Asn-171, Asn-332 and Asn-395 *N*-glycans are complex types, and those of Asn-402 and Asn-563 *N*-glycans are oligo-mannose types. A total of 3.4 % of the IgM in human serum contains $GlcNAc_2Man_{5\text{-}9}$. Of the *N*-glycans in IgM, 100 % of Asn-402 and 17 % of Asn-563 are $GlcNAc_2Man_{5–9}$. Through the binding of MBL to oligo-mannose and glycans terminated by GlcNAc in *N*-glycans, IgM molecules are capable of activating complement through the MBL pathway [21]. The Delta chain contains Asn-354, Asn-445 and Asn-496 three *N*-glycosylation sites and Ser-109, Ser-126, Ser-127, Ser-131, Ser-132 five *O*-glycosylation sites in the hinge region [21]. The Epsilon chain contains Asn-140, Asn-168, Asn-218, Asn-265, Asn-371, Asn-383 and Asn-394 seven *N*-glycosylation sites [22]. To date, little is known about the biological function of glycosylation in these Igs.

Effcet of glycosylation on Ig functionality

Effect of glycosylation on IgG functionality

IgG antibodies bind antigen to neutralize them and promote antibody-dependent cytotoxicity, opsonization, and phagocytosis. While antibody specificity is determined by the Fab portion, antibody effector functions are triggered by the Fc domain. These effector functions are heavily dependent on the Asn297 biantaennary glycan. This glycan plays a critical

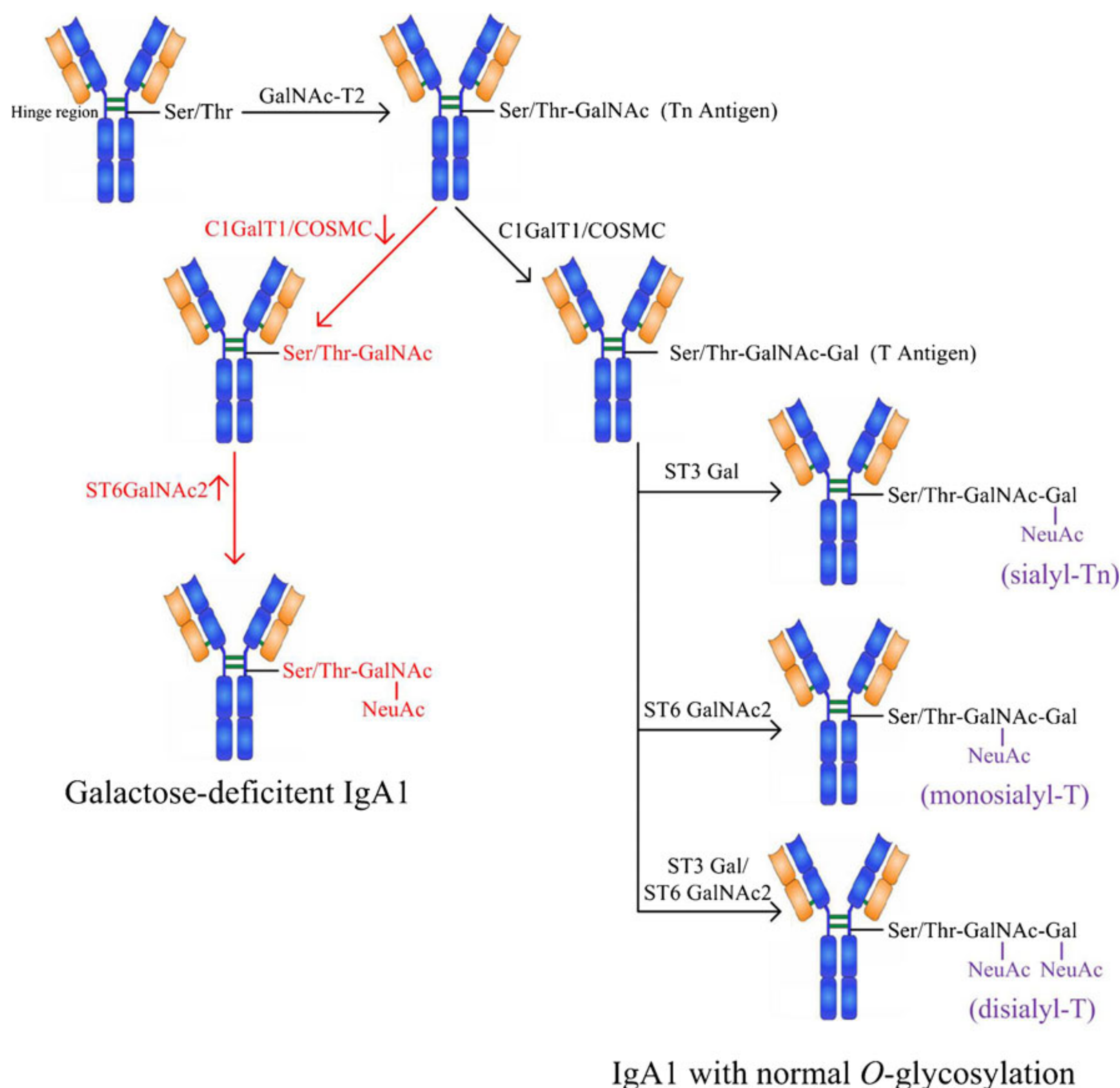

Fig. 2 *O*-glycosylation and aberrant *O*-glycosylation with galactose-deficiency of IgA1. The initial linking of GalNAc to the hinge region of IgA1 to form Tn antigen is catalyzed by one member of the UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetyl galactosaminyltransferase family, known as GalNAc-T2. Gal may be linked to the GalNAc by a core-1 β1,3-galactosyltransferase, known as C1GalT1, to form the disaccharide Gal-β-1,3-GalNAc (T antigen). This is cooperated by the chaperone protein core-1 β3-Gal-T-specific molecular chaperone (Cosmc). The Gal chain may be extended with one or two sialic acid units that are added during the catalysis of Gal: α2,3-sialyltarnsferase (ST3 Gal) or GalNAc: α2,6-sialyltransferase (ST6 GalNAc2) to form sialyl-Tn, monosialyl-T or disialyl-T. It is postulated that the down-regulation of C1GalT1/Cosmc and the up-regulation of ST6GalNAc2 may contribute to the hypo-galactosylation of IgA1 during IgAN (the left side of the figure, shown in red)

role in antibody stability and is believed to maintain the two heavy chains of Fc in an open conformation, which is required for interactions with activating FcγRs [23].

The current understanding of Fc glycosylation is based on studies with IgGs that are either fully-glycosylated (both heavy chains are glycosylated) or not glycosylated (neither heavy chain is glycosylated). Ha *et al.* compared the effect of hemi-glycosylation, with one heavy chain glycosylated and the other not glycosylated, on the stability and function of an IgG1 antibody compared to full glycosylation [24]. They found that neither Fab-mediated antigen binding nor FcRn receptor binding was affected by hemi-glycosylation. However, the thermal stability in the C_H2 domain of the hemi-glycosylated IgG1 was slightly decreased, and C1q binding to the antibody was also moderately decreased (approximately 20 %). More importantly, the hemi-glycosylated form showed significantly decreased binding affinities towards all Fc gamma receptors, including the high affinity FcγRI and the low affinity FcγRIIA, FcγRIIB, FcγRIIIA, and FcγRIIIB, resulting in a 3.5-fold decrease in ADCC activity.

Raju *et al.* recently reviewed the effects of terminal sugars of Fc glycans on IgG effector functions [25]. Asn297

N-glycan heterogeneity influenced both ADCC and CDC. Approximately 10 % of the Fc glycans in human serum IgG are sialylated, and non-sialylated Fc glycans are galactosylated with 0, 1 or 2 Gal residues at the terminals [26]. Hodoniczky *et al.* reported that increased Gal residues at the terminals of Fc glycans could enhance C1q binding to the antibody, resulting in enhanced CDC, but it did not influence antibody binding to FcγRIIIa, thus not affecting ADCC activity [27]. The IgG with GlcNAc at the terminals of Fc glycans is capable of binding MBL, consequently activating complement through the MBL pathway. In contrast, increased GlcNAc residues decreased C1q binding to the antibody [28]. In addition, it was reported that in the rapid initial phase of clearance, glycans carrying terminal *N*-acetylglucosamine (tGlcNAc) were selectively cleared from the circulation, and the sialic acid content and terminal galactose showed only small changes. These data confirmed the correlation of tGlcNAc to the half-life of the molecule and supported the hypothesis that the mannose receptor (which can also bind tGlcNAc) causes variable clearance of this molecule [29]. Asn297 *N*-glycan may contain a core fucose residue, which is transferred and linked to the reducing terminal GlcNAc by α1,6-fucosyltransferase from GDP-fucose [30]. It was observed that the lack of a core fucose in Asn297 *N*-glycan profoundly enhanced antibody affinity to FcγRIIIa, consequently enhancing ADCC [31]. It was recently reported that the defucosylation of an anti-HIV-1 neutralizing antibody 2G12 enhanced the antibody binding to FcγRIIIa, resulting in increased anti-HIV-1 activity [32]. The extent of sialylation of Asn297 *N*-glycan varies greatly. Decreased sialylation at the terminals of the glycan promotes IgG binding to FcγRIIIa, enhancing IgG ADCC and *vice versa* [33, 34]. Anthony and Ravetch found that enhanced sialylation of Asn297 *N*-glycan hindered IgG binding to FcγR, but it promoted IgG binding to the C-type lectins SIGN-R1 or DC-SIGN [23]. This initiated an anti-inflammatory cascade and upregulated the expression of inhibitory FcR and FcγRIIb on the surface of inflammatory cells, consequently dampening autoantibody-mediated inflammation [35]. They observed that the anti-inflammatory activity of intravenous immunoglobulin (IVIG) was influenced by α2-6-sialic acid residues linked to the galactose at the terminal of the Fc glycans. The effector functions of IgG are also affected by high mannose structures. Human monoclonal antibodies produced in the presence of kifunensine contained mainly oligomannose-type glycans, and they expressed increased ADCC activity and affinity for FcγRIIIA but reduced C1q binding [36].

Fc *N*-glycan plays an important role in the binding of Fc to the Fc receptor. Fc aglycosylation results in a 15-20-fold decrease in IgG1 binding to FcγRIII [37]. In a study of the binding of an IgG1 antibody (cetuximab) with ADCC activity to the epithelial growth factor receptor on tumor cells, Peter *et al.* found that aglycosylated cetuximab did not bind to FcγRI or FcγRIIIa, nor did it have ADCC activity even at high effector:target cell ratios [38]. Indeed, the binding of Fc to its Fc receptor is quite sensitive to Asn297 *N*-glycosylation. Aglycosylation of Asn297 by site-directed mutagenesis [39] or enzyme treatment [40] resulted in the loss of binding of IgG Fc to the FcγR with low affinity. This phenomenon limits the use of therapeutic antibodies in the clinic because therapeutic activity is based on the binding of the antibody Fc to its Fc receptor on immune cells. IgG Fc binding to FcγR is based on both the interaction between proteins and the interaction between FcγR protein and Fc *N*-glycan [25]. Sazinsky *et al.* demonstrated that not glycosylated Fcs resulting from substitutions at both N297 and T299 of the glycosylation motif could maintain engagement of FcγRs, suggesting a possibility of engineering therapeutic antibodies with Fc-effector functions without glycosylating the antibodies [41]. In a recent review, Anthony and Nimmerjahn summarized the discoveries of the role of differential IgG glycosylation on the interaction of antibodies with FcγRs *in vivo* [35]. They said that the identification of the IgG-bound sugar moiety as an important modulator of IgG activity made it an attractive target for selective potentiation of either the pro-inflammatory or the anti-inflammatory activities of IgG. These findings would provide optimized IgG-based therapeutics to either enhance IgG-dependent tumor cell destruction or suppress IgG-dependent autoimmunity and organ rejection.

Effect of glycosylation on IgA functionality

In examining aberrant *O*-glycosylation in the hinge region of IgA1 during IgAN nephropathy, galactosylation was decreased in the *O*-glycans, resulting in an increase of truncated *O*-glycans with terminal GalNAc or sialylated GalNAc [42, 43]. Hypo-galactosylated IgA1 is prone to self-aggregation and forming immune complexes with the IgG antibody against the hinge region of IgA1 [43, 44]. *In vitro* experiments showed that hypo-galactosylated IgA1 had increased affinity for extracellular matrix proteins, such as fibronectin and IV type collagen, leading to the deposition of circulating IgA1 immune complexes in mesangial cells [43, 44]. In addition, hypo-galactosylated IgA1 was capable of inducing the proliferation and apoptosis of mesangial cells [45], downregulating the synthesis and secretion of vascular endothelial growth factor [46], affecting integrin expression in human mesangial cells [47], and promoting the synthesis of extracellular matrix proteins. The immune complexes and the aggregates containing hypo-galactosylated IgA1 were capable of activating complement [48]. In contrast to that of IgG, *N*-glycosylation of IgA1 did not affect Fc binding to FcαR1 [49].

Effect of glycosylation on other Ig functionality

To date, few papers have examined the effect of glycosylation on the properties and functions of IgM, IgE and IgD. IgM

contains an Asn406 *N*-glycosylation site. Wright *et al.* observed that *N*-glycosylation at Asn402 resulting from the mutation of Asn406 to Ser decreased C1q binding by 12-fold [50]. Bjorklundet *et al.* reported that *N*-glycosylation influenced epitope expression and the receptor binding structure in human IgE [51]. It was previously observed that the IgD synthesized by B cells was not *N*-glycosylated in the presence of tunicamycin and could not be secreted by the cells. However, Gala and Morrison reported that only the *N*-glycan nearest the disulphide bond between the heavy chains played an important role in IgD secretion, and *O*-glycosylation did not affect IgD secretion [52].

Aberrant Ig glycosylation and disease

Aberrant IgG glycosylation and disease

With advances in our understanding of the biological significance of glycosylation, the importance of aberrant glycosylation in disease development is becoming clearer. Aberrant IgG glycosylation has been associated with diseases, such as ANCA-associated systemic vasculitis [53], periodontal disease [54], ovarian cancer [55, 56], Lambert-Eaton myasthenic syndrome and myasthenia gravis [57], fetal-maternal alloimmune thrombocytopenia [58], and multiple myeloma [59]. Here, we use rheumatoid arthritis (RA) to illuminate the effects of aberrant IgG glycosylation on disease pathogenesis (Table 1).

Parekh *et al.* reported for the first time in 1985 that the *N*-glycan of IgG in the serum and synovial fluid of RA patients was highly agalactosyl IgG [7]. They then observed a close association between the extent of agalactosylation of IgG *N*-glycan and the onset of adult and juvenile RA patients [60]. In the remission of arthritis during pregnancy, the extent of agalactosylation of IgG *N*-glycan was reduced [11]. Croce *et al.* examined the possible mechanisms of the monoclonal antibody infliximab, which showed a therapeutic effect on RA, and found a profound decrease of agalactosyl IgG in the patients with disease remission treated with infliximab [61]. Recently, Ercan *et al.* reported that aberrant IgG galactosylation beginning prior to disease onset was associated with disease activity in RA patients, especially in women, and it resided preferentially in autoantibodies [62].

Because galactose is transferred to *N*-glycans in proteins by β1,4galactosyltransferase (β4GalT) to extend saccharide chains, Axfold *et al.* examined the relationship between agalactosylation in serum IgG and lymphocyte β4GalT [63]. They observed that while β4GalT activity decreased during RA, the amount of agalactosyl IgG increased, and the relationship was linear. In contrast, both β4GalT activity and agalactosyl IgG were increased in healthy controls. In addition, they found anti-β4GalT IgG in RA patients. They postulated that the anti-β4GalT IgG was deactivating β4GalT, resulting in agalactosyl IgG during RA development. The terminal of the agalactosyl IgG in RA is GlcNAc, which is capable of binding to MBL and activating complement. This suggests the possibility that synovial membrane inflammation

Table 1 Glycan changes in diseases

Condition	*N*-/*O*-glycan changes	References
(IgG)		
Rheumatoid arthritis	Decrease in Gal in *N*-glycans	[7, 11, 28, 60–64]
ANCA-associated systemic vasculitis	Decrease in Gal in *N*-glycans	[53]
Periodontal disease	Decrease in Gal in *N*-glycans	[54]
Ovarian cancer	Increase in Man in *N*-glycans	[55]
	Increase in Fuc and NeuAc, decrease in Gal in *N*-glycans	[56]
Lambert-Eaton myasthenic syndrome	Decrease in Gal in *N*-glycans	[57]
Myasthenia gravis	Decrease in Gal in *N*-glycans	[57]
Fetal-maternal alloimmune thrombocytopenia	Decrease in Fuc, increase in Gal and NeuAc in *N*-glycans	[58]
Multiple myeloma	Decrease in Gal, stage-dependent changes in NeuAc in *N*-glycans	[59]
(IgA)		
IgA nephropathy	Decrease in Gal in *O*-glycans	[43–45, 65–71, 78, 80, 85, 86]
Henoch-Schonlein purpura	Decrease in Gal in *O*-glycans	[86, 94]
Sjogren syndrome	Decrease in Gal and increase in NeuAc in *O*-glycans	[95]

during RA may partially result from complement activation through the MBL pathway induced by accumulated agalactosyl IgG in the joints [28]. Troelson *et al.* observed that decreased galactosylation of IgG in RA patients correlated to markers of inflammation, such as IL-6 and CRP, and IgG hypo-galactosylation was associated with markers of joint destruction only in patients homozygous for the MBL2 genotype YA/YA. This suggested that inflammation-associated decreased galactosylation of IgG combined with highly expressed MBL2 genotypes are involved in the pathophysiology of RA [64].

Aberrant IgA glycosylation and disease

IgA nephropathy (IgAN), described in 1968 as an IgA-IgG immune-complex disease, is an autoimmune disease. During IgAN, galactose-deficient IgA1 is recognized by unique autoantibodies, resulting in the formation of pathogenic immune complexes that ultimately cause glomerular injury. Thus, the formation of galactose-deficient IgA1-containing immune complexes is a critical factor for the pathogenesis of IgA nephropathy. Although the exact pathogenesis of IgAN is still poorly defined, it is well accepted that IgAN pathogenesis covers four links: aberrant *O*-glycosylation of IgA1 on the heavy-chain hinge region with galactose deficiency, the induction of circulating IgG or IgA autoantibodies specific for galactose-deficient IgA1, the formation of nephritogenic immune complexes and their deposition in the glomerular mesangium, and the induction of a local proliferative and inflammatory response of mesangial cells by the immune complexes.

As mentioned previously, IgA1 is normally *O*-glycosylated with β1-3-linked galactose in the hinge region. However, the *O*-linked glycans in the majority of IgA1 in the glomerular deposits in patients with IgAN are galactose-deficient [65–68]. This aberrantly glycosylated IgA1 is also detected in the sera and in circulating immune complexes in IgAN patients, while it is rarely found in healthy controls [65]. Because galactose is attached to GalNAc by core-1 β1,3-galactosyltransferase (C1GalT1) coordinated with chaperone Cosmc, and sialic acid (NeuAc) is attached to GalNAc by α2,6-GalNAc-sialyltransferase2 (ST6GalNAc2), it can been hypothesized that the imbalance in the activities of C1GalT1/COSMC and ST6GalNAc2 contribute to the hypo-galactosylation of IgA1 during IgAN (Fig 2). A recent *in vitro* study by Suzuki *et al.* showed that C1GalT1 activity was significantly lower in EBV-immortalized IgA-secreting lymphocytes from patients with IgAN, while ST6GalNAc2 activity was significantly higher, supporting this hypothesis [69].

During the induction of IgAN, galactose-deficient IgA1 combines with IgG or IgA autoantibodies that are specific to galactose-deficient glycans on the hinge region of IgA1 to form immune complexes [65]. Galactose-deficient IgA1 is prone to self-aggregation, the formation of IgA1-IgA1 complexes and reactivity with IgG or IgA autoantibodies [43, 44, 70]. In a recent study on the association of serum levels of IgG and IgA autoantibodies with the progression of IgAN nephropathy, Berthoux *et al.* found that the mean serum levels of autoantibodies targeting galactose-deficient IgA1 were significantly higher in patients than in controls, and increasing levels of these autoantibodies correlated with worse clinical outcomes [71]. Deposition of immune complexes in the mesangium in the kidney possibly induces the production of inflammatory mediators by mesangial cells, such as interleukin-6 [72, 73], transforming growth factor β1 [74–76], and platelet-derived growth factor [77], as levels of these mediators were higher in the serum of IgAN patients than in normal controls. These cytokines were associated with mesangial proliferation or tubulointerstitial fibrosis. It was recently demonstrated that circulating immune complexes containing galactose-deficient IgA1 prepared from the sera of IgAN patients could stimulate mesangial cell proliferation *in vitro* [45]. A possible pathologic role of the complement pathway during IgAN was recently examined in two IgAN-susceptible mouse models. It was first demonstrated that galactose-deficient IgA1-containing immune complexes induced complement activation, leading to the full progression of IgAN [78]. While examining the molecular mechanisms involved in the progression of IgAN, Tamouza *et al.* observed that the MAPK/ERK signaling pathway was activated in the mesangium of several patients [79]. They also found that circulating large-molecular-mass IgA1-containing immune complexes purified from patient sera were able to activate ERK in human mesangial cells *in vitro*, and pro-inflammatory cytokine secretion by these cells was controlled by ERK activation through mesangial IgA1 receptor (CD71).

Hereditary factors may play an important role in IgAN pathogenesis. The observation of increased galactose-deficient IgA1 levels in the serum of patients with IgAN and their relatives compared to healthy controls supports the genetic predisposition of aberrant IgA glycosylation [80]. Family type IgAN has been found in various parts of the world [81–84]. Colleen Hastings *et al.* [85] and Kiryluk *et al.* [86] recently reported that serum galactose-deficient IgA1 was an inherited risk factor for adult IgAN. Kiryluk *et al.* also observed that galactose-deficient IgA1 levels in the serum were highly inherited in pediatric patients with IgAN. However, it is difficult to identify the genes responsible for galactose-deficiency of IgA1. It is possible that the genes associated with IgAN pathogenesis will be identified through whole genome analysis [87]. It is strongly postulated that IgAN pathogenesis results from the interaction of hereditary and environmental factors.

Although alterations in IgA1 glycosylation certainly play an important role in the progression of kidney disease in IgAN, serum levels of galactose-deficient IgA1 are not elevated in a significant proportion of patients with IgAN [85, 88], and relatives with higher serum levels of aberrantly glycosylated IgA1 were mostly asymptomatic. This suggests that additional cofactors other than aberrant IgA glycosylation may be required for the development of IgAN [80]. Clinically, IgAN may be punctuated by episodes of macroscopic hematuria with pharingotonsillitis, or it may be oligosymptomatic with microscopic hematuria and mild proteinuria. For example, IgAN development is often promoted by upper respiratory tract infections, especially acute tonsillitis [89]. Tonsillectomy may have a beneficial effect on the prevention of renal dysfunction in patients with IgAN [89], a delay of IgAN progression [90, 91], and a promotion of clinical remission. Furthermore, serum levels of aggregated IgA1 are markedly reduced after tonsillectomy.

Tonsillitis is often caused by viruses, such as *Haemophilus parainfluenzae*, or bacterial infections. Microbes, including viruses and bacteria, induce immune responses, and DNA is one of the microbial immunogens capable of doing so. It is well known that the microbial genome contains high levels of CpG, and CpG-ODN has been used to mimic microbial DNA to induce immune response *in vitro*. Goto *et al.* [92] observed increased spontaneous production of IgA and IFN-γ by tonsil mononuclear cells from IgAN patients in comparison to those from healthy controls. Tonsil mononuclear cells from IgAN patients secreted higher levels of IgA, BAFF and IFN-γ upon stimulation with CpG-ODN. In the presence of anti-BAFF or anti-IFN-γ antibody, CpG-ODN-driven IgA production by tonsil mononuclear cells was inhibited. However, in the presence of IFN-γ, BAFF production by tonsil mononuclear cells increased. These data suggest that the immune response to microbial DNA during acute tonsillitis may play a role in the pathogenesis of IgAN.

The data also suggest a bone marrow origin of the hypogalactosylated IgA1-producing B cells. However, Oortwijn *et al.* found secretary IgA, MBL and C4b in the multi-aggregated IgA deposited on the kidney mesangial membrane in some (15 %) of the IgAN patients, suggesting a possibility of aberrant mucosal immunity in IgAN pathogenesis [72]. In a review, Novak *et al.* wrote that "an abnormal mucosal immune response resulting in production of galactose-deficient IgA1 in IgAN patients is supported by several observations: the aberrant glycosylation affects mostly polymeric IgA1 produced by mucosal-associated IgA1-secreting cells (including those from tonsils), the synpharyngitic nature of the macroscopic hematuria, and the association of disease severity with polymorphisms of a pattern-recognition receptor, TLR9". Novak *et al.* postulated that the aberrant glycosylation of IgA1 may reflect abnormal mucosal immune responses to infections of the upper respiratory tract in genetically predisposed individuals [93].

In addition to IgAN, Henoch-Schonlein purpura [86, 94] and Sjögren syndrome [95] were also associated with aberrant *O*-glycosylation of IgA (Table 1).

Concluding remarks

Glycans in Igs are heterogeneous, and this heterogeneity influences antibody stability, binding to antigens on the cell surface, and effector functions. Aberrant glycosylation of Igs has been associated with several diseases. It has also been observed that Igs with special glycosylation patterns have therapeutic effects on certain diseases. For example, intravenous sialylated IgG has anti-inflammatory effects. However, our knowledge of Ig glycosylation in health and disease remains quite limited. We believe that more diseases will be found to be associated with aberrant Ig glycosylation, and the related mechanisms will be discovered in the future. In addition, efficient therapeutics to combat these diseases will be developed.

In addition to Ig glycosylation, FcR glycosylation also affects the interaction between Ig Fc and its respective receptors. For example, we recently discovered that removal of the N-glycosylation site at N58 of FcαR (CD89) by site-directed mutation resulted in a marked increase of IgA binding, and desialylation of N58 contributed to the increased binding [96]. Thus, in studies of the effect of glycosylation on antibody effector functions, the effect of Fc receptor glycosylation on the interaction between Ig Fc and FcR should also be examined.

Acknowledgments This work was supported by a grant (2012 J24) from PUMC Youth Fund and a grant (DWS201208) from the Fundamental Research Funds for the Central Universities of China.

References

1. Skropeta, D.: The effect of individual N-glycans on enzyme activity. Bioorg. Med. Chem. **17**(7), 2645–2653 (2009). doi:10.1016/j.bmc.2009.02.037
2. Ohtsubo, K., Marth, J.D.: Glycosylation in cellular mechanisms of health and disease. Cell **126**(5), 855–867 (2006). doi:10.1016/j.cell.2006.08.019
3. Dennis, J.W., Lau, K.S., Demetriou, M., Nabi, I.R.: Adaptive regulation at the cell surface by N-glycosylation. Traffic **10**(11), 1569–1578 (2009). doi:10.1111/j.1600-0854.2009.00981.x
4. Marth, J.D., Grewal, P.K.: Mammalian glycosylation in immunity. Nature reviews. Immunology **8**(11), 874–887 (2008). doi:10.1038/nri2417
5. Arnold, J.N., Wormald, M.R., Sim, R.B., Rudd, P.M., Dwek, R.A.: The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu. Rev. Immunol. **25**, 21–50 (2007). doi:10.1146/annurev.immunol.25.022106.141702
6. Mizuochi, T., Taniguchi, T., Shimizu, A., Kobata, A.: Structural and numerical variations of the carbohydrate moiety of immunoglobulin G. J. Immunol. **129**(5), 2016–2020 (1982)

7. Parekh, R.B., Dwek, R.A., Sutton, B.J., Fernandes, D.L., Leung, A., Stanworth, D., Rademacher, T.W., Mizuochi, T., Taniguchi, T., Matsuta, K., et al.: Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature **316**(6027), 452–457 (1985)
8. Parekh, R., Roitt, I., Isenberg, D., Dwek, R., Rademacher, T.: Age-related galactosylation of the N-linked oligosaccharides of human serum IgG. J. Experim. Med. **167**(5), 1731–1736 (1988)
9. Yamada, E., Tsukamoto, Y., Sasaki, R., Yagyu, K., Takahashi, N.: Structural changes of immunoglobulin G oligosaccharides with age in healthy human serum. Glycoconj. J. **14**(3), 401–405 (1997)
10. Ruhaak, L.R., Uh, H.W., Beekman, M., Koeleman, C.A., Hokke, C.H., Westendorp, R.G., Wuhrer, M., Houwing-Duistermaat, J.J., Slagboom, P.E., Deelder, A.M.: Decreased levels of bisecting GlcNAc glycoforms of IgG are associated with human longevity. PLoS One **5**(9), e12566 (2010). doi:10.1371/journal.pone.0012566
11. Rook, G.A., Steele, J., Brealey, R., Whyte, A., Isenberg, D., Sumar, N., Nelson, J.L., Bodman, K.B., Young, A., Roitt, I.M., et al.: Changes in IgG glycoform levels are associated with remission of arthritis during pregnancy. J. Autoimmun. **4**(5), 779–794 (1991)
12. Kelleher, D.J., Karaoglu, D., Mandon, E.C., Gilmore, R.: Oligosaccharyltransferase isoforms that contain different catalytic STT3 subunits have distinct enzymatic properties. Mol. Cell **12**(1), 101–111 (2003)
13. Prados, M.B., La Blunda, J., Szekeres-Bartho, J., Caramelo, J., Miranda, S.: Progesterone induces a switch in oligosaccharyltransferase isoform expression: consequences on IgG N-glycosylation. Immunol. Lett. **137**(1–2), 28–37 (2011). doi:10.1016/j.imlet.2011.01.017
14. Yoo, E.M., Morrison, S.L.: IgA: an immune glycoprotein. Clin. Immunol. **116**(1), 3–10 (2005). doi:10.1016/j.clim.2005.03.010
15. Takahashi, K., Hiki, Y., Odani, H., Shimozato, S., Iwase, H., Sugiyama, S., Usuda, N.: Structural analyses of O-glycan sugar chains on IgA1 hinge region using SELDI-TOFMS with various lectins. Biochem. Biophys. Res. Commun. **350**(3), 580–587 (2006). doi:10.1016/j.bbrc.2006.09.075
16. Iwasaki, H., Zhang, Y., Tachibana, K., Gotoh, M., Kikuchi, N., Kwon, Y.D., Togayachi, A., Kudo, T., Kubota, T., Narimatsu, H.: Initiation of O-glycan synthesis in IgA1 hinge region is determined by a single enzyme, UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2. J. Biol. Chem. **278**(8), 5613–5621 (2003). doi:10.1074/jbc.M211097200
17. Ju, T., Brewer, K., D'Souza, A., Cummings, R.D., Canfield, W.M.: Cloning and expression of human core 1 beta1,3-galactosyltransferase. J. Biol. Chem. **277**(1), 178–186 (2002). doi:10.1074/jbc.M109060200
18. Ju, T., Cummings, R.D.: A unique molecular chaperone Cosmc required for activity of the mammalian core 1 beta 3-galactosyltransferase. Proc. Natl. Acad. Sci. U. S. A. **99**(26), 16613–16618 (2002). doi:10.1073/pnas.262438199
19. Hiki, Y.: O-linked oligosaccharides of the IgA1 hinge region: Roles of its aberrant structure in the occurrence and/or progression of IgA nephropathy. Clin. Exp. Nephrol. **13**(5), 415–423 (2009). doi:10.1007/s10157-009-0173-7
20. Mestecky, J., Tomana, M., Moldoveanu, Z., Julian, B.A., Suzuki, H., Matousovic, K., Renfrow, M.B., Novak, L., Wyatt, R.J., Novak, J.: Role of aberrant glycosylation of IgA1 molecules in the pathogenesis of IgA nephropathy. Kid. Blood Press. Res. **31**(1), 29–37 (2008). doi:10.1159/000112922
21. Arnold, J.N., Wormald, M.R., Suter, D.M., Radcliffe, C.M., Harvey, D.J., Dwek, R.A., Rudd, P.M., Sim, R.B.: Human serum IgM glycosylation: Identification of glycoforms that can bind to mannan-binding lectin. J. Biol. Chem. **280**(32), 29080–29087 (2005). doi:10.1074/jbc.M504528200
22. Arnold, J.N., Radcliffe, C.M., Wormald, M.R., Royle, L., Harvey, D.J., Crispin, M., Dwek, R.A., Sim, R.B., Rudd, P.M.: The glycosylation of human serum IgD and IgE and the accessibility of identified oligomannose structures for interaction with mannan-binding lectin. J. Immunol. **173**(11), 6831–6840 (2004)
23. Anthony, R.M., Ravetch, J.V.: A novel role for the IgG Fc glycan: The anti-inflammatory activity of sialylated IgG Fcs. J. Clin. Immunol. **30**(Suppl 1), S9–14 (2010). doi:10.1007/s10875-010-9405-6
24. Ha, S., Ou, Y., Vlasak, J., Li, Y., Wang, S., Vo, K., Du, Y., Mach, A., Fang, Y., Zhang, N.: Isolation and characterization of IgG1 with asymmetrical Fc glycosylation. Glycobiology **21**(8), 1087–1096 (2011). doi:10.1093/glycob/cwr047
25. Raju, T.S.: Terminal sugars of Fc glycans influence antibody effector functions of IgGs. Curr. Opin. Immunol. **20**(4), 471–478 (2008). doi:10.1016/j.coi.2008.06.007
26. Flynn, G.C., Chen, X., Liu, Y.D., Shah, B., Zhang, Z.: Naturally occurring glycan forms of human immunoglobulins G1 and G2. Mol. Immunol. **47**(11–12), 2074–2082 (2010). doi:10.1016/j.molimm.2010.04.006
27. Hodoniczky, J., Zheng, Y.Z., James, D.C.: Control of recombinant monoclonal antibody effector functions by Fc N-glycan remodeling *in vitro*. Biotechnol. Prog. **21**(6), 1644–1652 (2005). doi:10.1021/bp050228w
28. Malhotra, R., Wormald, M.R., Rudd, P.M., Fischer, P.B., Dwek, R.A., Sim, R.B.: Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement *via* the mannose-binding protein. Nat. Med. **1**(3), 237–243 (1995)
29. Jones, A.J., Papac, D.I., Chin, E.H., Keck, R., Baughman, S.A., Lin, Y.S., Kneer, J., Battersby, J.E.: Selective clearance of glycoforms of a complex glycoprotein pharmaceutical caused by terminal N-acetylglucosamine is similar in humans and cynomolgus monkeys. Glycobiology **17**(5), 529–540 (2007). doi:10.1093/glycob/cwm017
30. Miyoshi, E., Noda, K., Yamaguchi, Y., Inoue, S., Ikeda, Y., Wang, W., Ko, J.H., Uozumi, N., Li, W., Taniguchi, N.: The alpha1-6-fucosyltransferase gene and its biological significance. Biochim. Biophys. Acta **1473**(1), 9–20 (1999)
31. Satoh, M., Iida, S., Shitara, K.: Non-fucosylated therapeutic antibodies as next-generation therapeutic antibodies. Expert. Opin. Biol. Ther. **6**(11), 1161–1173 (2006). doi:10.1517/14712598.6.11.1161
32. Forthal, D.N., Gach, J.S., Landucci, G., Jez, J., Strasser, R., Kunert, R., Steinkellner, H.: Fc-glycosylation influences Fcgamma receptor binding and cell-mediated anti-HIV activity of monoclonal antibody 2G12. J. Immunol. **185**(11), 6876–6882 (2010). doi:10.4049/jimmunol.1002600
33. Scallon, B.J., Tam, S.H., McCarthy, S.G., Cai, A.N., Raju, T.S.: Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality. Mol. Immunol. **44**(7), 1524–1534 (2007). doi:10.1016/j.molimm.2006.09.005
34. Kaneko, Y., Nimmerjahn, F., Ravetch, J.V.: Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science **313**(5787), 670–673 (2006). doi:10.1126/science.1129594
35. Anthony, R.M., Nimmerjahn, F.: The role of differential IgG glycosylation in the interaction of antibodies with FcgammaRs *in vivo*. Curr. Opin. Org Transplant. **16**(1), 7–14 (2011). doi:10.1097/MOT.0b013e328342538f
36. Zhou, Q., Shankara, S., Roy, A., Qiu, H., Estes, S., McVie-Wylie, A., Culm-Merdek, K., Park, A., Pan, C., Edmunds, T.: Development of a simple and rapid method for producing non-fucosylated oligomannose containing antibodies with increased effector function. Biotechnol. Bioeng. **99**(3), 652–665 (2008). doi:10.1002/bit.21598
37. Radaev, S., Sun, P.D.: Recognition of IgG by Fcgamma receptor. The role of Fc glycosylation and the binding of peptide inhibitors. J. Biol. Chem. **276**(19), 16478–16483 (2001). doi:10.1074/jbc.M100351200
38. Patel, D., Guo, X., Ng, S., Melchior, M., Balderes, P., Burtrum, D., Persaud, K., Luna, X., Ludwig, D.L., Kang, X.: IgG isotype, glycosylation, and EGFR expression determine the induction of

antibody-dependent cellular cytotoxicity *in vitro* by cetuximab. Human Anti. **19**(4), 89–99 (2010). doi:10.3233/HAB-2010-0232
39. Tao, M.H., Morrison, S.L.: Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. J. Immunol. **143**(8), 2595–2601 (1989)
40. Mimura, Y., Sondermann, P., Ghirlando, R., Lund, J., Young, S.P., Goodall, M., Jefferis, R.: Role of oligosaccharide residues of IgG1-Fc in Fc gamma RIIb binding. J. Biol. Chem. **276**(49), 45539–45547 (2001). doi:10.1074/jbc.M107478200
41. Sazinsky, S.L., Ott, R.G., Silver, N.W., Tidor, B., Ravetch, J.V., Wittrup, K.D.: Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. Proc. Natl. Acad. Sci. U. S. A. **105**(51), 20167–20172 (2008). doi:10.1073/pnas.0809257105
42. Shimozato, S., Hiki, Y., Odani, H., Takahashi, K., Yamamoto, K., Sugiyama, S.: Serum under-galactosylated IgA1 is increased in Japanese patients with IgA nephropathy. Nephrology, Dialysis, Transplantation: Official publication of the European Dialysis and Transplant Association - European Renal Association **23**(6), 1931–1939 (2008). doi:10.1093/ndt/gfm913
43. Kokubo, T., Hiki, Y., Iwase, H., Tanaka, A., Toma, K., Hotta, K., Kobayashi, Y.: Protective role of IgA1 glycans against IgA1 self-aggregation and adhesion to extracellular matrix proteins. J. Am. Soc. Nephrol. **9**(11), 2048–2054 (1998)
44. Yan, Y., Xu, L.X., Zhang, J.J., Zhang, Y., Zhao, M.H.: Self-aggregated deglycosylated IgA1 with or without IgG were associated with the development of IgA nephropathy. Clin. Exp. Immunol. **144**(1), 17–24 (2006). doi:10.1111/j.1365-2249.2006.03026.x
45. Novak, J., Tomana, M., Matousovic, K., Brown, R., Hall, S., Novak, L., Julian, B.A., Wyatt, R.J., Mestecky, J.: IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells. Kidney Int. **67**(2), 504–513 (2005). doi:10.1111/j.1523-1755.2005.67107.x
46. Amore, A., Conti, G., Cirina, P., Peruzzi, L., Alpa, M., Bussolino, F., Coppo, R.: Aberrantly glycosylated IgA molecules downregulate the synthesis and secretion of vascular endothelial growth factor in human mesangial cells. Amer. J. Kidney Dis.: Off. J. Nat. Kidney Found. **36**(6), 1242–1252 (2000). doi:10.1053/ajkd.2000.19840
47. Peruzzi, L., Amore, A., Cirina, P., Trusolino, L., Basso, G., Ricotti, E., Emancipator, S.N., Marchisio, P.C., Coppo, R.: Integrin expression and IgA nephropathy: *In vitro* modulation by IgA with altered glycosylation and macromolecular IgA. Kidney Int. **58**(6), 2331–2340 (2000). doi:10.1046/j.1523-1755.2000.00417.x
48. Zhang, W., Lachmann, P.J.: Glycosylation of IgA is required for optimal activation of the alternative complement pathway by immune complexes. Immunology **81**(1), 137–141 (1994)
49. Gomes, M.M., Wall, S.B., Takahashi, K., Novak, J., Renfrow, M.B., Herr, A.B.: Analysis of IgA1 N-glycosylation and its contribution to FcalphaRI binding. Biochemistry **47**(43), 11285–11299 (2008). doi:10.1021/bi801185b
50. Wright, J.F., Shulman, M.J., Isenman, D.E., Painter, R.H.: C1 binding by mouse IgM. The effect of abnormal glycosylation at position 402 resulting from a serine to asparagine exchange at residue 406 of the mu-chain. J. Biol. Chem. **265**(18), 10506–10513 (1990)
51. Bjorklund, J.E., Karlsson, T., Magnusson, C.G.: N-glycosylation influences epitope expression and receptor binding structures in human IgE. Mol. Immunol. **36**(3), 213–221 (1999)
52. Gala, F.A., Morrison, S.L.: The role of constant region carbohydrate in the assembly and secretion of human IgD and IgA1. J. Biol. Chem. **277**(32), 29005–29011 (2002). doi:10.1074/jbc.M203258200
53. Holland, M., Yagi, H., Takahashi, N., Kato, K., Savage, C.O., Goodall, D.M., Jefferis, R.: Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA-associated systemic vasculitis. Biochim. Biophys. Acta **1760**(4), 669–677 (2006). doi:10.1016/j.bbagen.2005.11.021
54. Novak, J., Tomana, M., Shah, G.R., Brown, R., Mestecky, J.: Heterogeneity of IgG glycosylation in adult periodontal disease. J. Dent. Res. **84**(10), 897–901 (2005)
55. Gercel-Taylor, C., Bazzett, L.B., Taylor, D.D.: Presence of aberrant tumor-reactive immunoglobulins in the circulation of patients with ovarian cancer. Gynecol. Oncol. **81**(1), 71–76 (2001). doi:10.1006/gyno.2000.6102
56. Saldova, R., Royle, L., Radcliffe, C.M., Abd Hamid, U.M., Evans, R., Arnold, J.N., Banks, R.E., Hutson, R., Harvey, D.J., Antrobus, R., Petrescu, S.M., Dwek, R.A., Rudd, P.M.: Ovarian cancer is associated with changes in glycosylation in both acute-phase proteins and IgG. Glycobiology **17**(12), 1344–1356 (2007). doi:10.1093/glycob/cwm100
57. Selman, M.H., Niks, E.H., Titulaer, M.J., Verschuuren, J.J., Wuhrer, M., Deelder, A.M.: IgG fc N-glycosylation changes in Lambert-Eaton myasthenic syndrome and myasthenia gravis. J. Prot. Res. **10**(1), 143–152 (2011). doi:10.1021/pr1004373
58. Wuhrer, M., Porcelijn, L., Kapur, R., Koeleman, C.A., Deelder, A., de Haas, M., Vidarsson, G.: Regulated glycosylation patterns of IgG during alloimmune responses against human platelet antigens. J. Prot. Res. **8**(2), 450–456 (2009). doi:10.1021/pr800651j
59. Aurer, I., Lauc, G., Dumic, J., Rendic, D., Matisic, D., Milos, M., Heffer-Lauc, M., Flogel, M., Labar, B.: Aberrant glycosylation of Igg heavy chain in multiple myeloma. Colleg. Antropolog. **31**(1), 247–251 (2007)
60. Parekh, R.B., Roitt, I.M., Isenberg, D.A., Dwek, R.A., Ansell, B.M., Rademacher, T.W.: Galactosylation of IgG associated oligosaccharides: Reduction in patients with adult and juvenile onset rheumatoid arthritis and relation to disease activity. Lancet **1**(8592), 966–969 (1988)
61. Croce, A., Firuzi, O., Altieri, F., Eufemi, M., Agostino, R., Priori, R., Bombardieri, M., Alessandri, C., Valesini, G., Saso, L.: Effect of infliximab on the glycosylation of IgG of patients with rheumatoid arthritis. J. Clin. Lab. Anal. **21**(5), 303–314 (2007). doi:10.1002/jcla.20191
62. Ercan, A., Cui, J., Chatterton, D.E., Deane, K.D., Hazen, M.M., Brintnell, W., O'Donnell, C.I., Derber, L.A., Weinblatt, M.E., Shadick, N.A., Bell, D.A., Cairns, E., Solomon, D.H., Holers, V.M., Rudd, P.M., Lee, D.M.: Aberrant IgG galactosylation precedes disease onset, correlates with disease activity, and is prevalent in autoantibodies in rheumatoid arthritis. Arthr. Rheum. **62**(8), 2239–2248 (2010). doi:10.1002/art.27533
63. Axford, J.S., Sumar, N., Alavi, A., Isenberg, D.A., Young, A., Bodman, K.B., Roitt, I.M.: Changes in normal glycosylation mechanisms in autoimmune rheumatic disease. J. Clin. Investig. **89**(3), 1021–1031 (1992). doi:10.1172/JCI115643
64. Troelsen, L.N., Jacobsen, S., Abrahams, J.L., Royle, L., Rudd, P.M., Narvestad, E., Heegaard, N.H., Garred, P.: IgG glycosylation changes and MBL2 polymorphisms: Associations with markers of systemic inflammation and joint destruction in rheumatoid arthritis. J. Rheumatol. **39**(3), 463–469 (2012). doi:10.3899/jrheum.110584
65. Galla, J.H.: IgA nephropathy. Kidney Int. **47**(2), 377–387 (1995)
66. Ponticelli, C., Traversi, L., Feliciani, A., Cesana, B.M., Banfi, G., Tarantino, A.: Kidney transplantation in patients with IgA mesangial glomerulonephritis. Kidney Int. **60**(5), 1948–1954 (2001). doi:10.1046/j.1523-1755.2001.00006.x
67. Hiki, Y., Odani, H., Takahashi, M., Yasuda, Y., Nishimoto, A., Iwase, H., Shinzato, T., Kobayashi, Y., Maeda, K.: Mass spectrometry proves under-O-glycosylation of glomerular IgA1 in IgA nephropathy. Kidney Int. **59**(3), 1077–1085 (2001). doi:10.1046/j.1523-1755.2001.0590031077.x
68. Gomes, M.M., Suzuki, H., Brooks, M.T., Tomana, M., Moldoveanu, Z., Mestecky, J., Julian, B.A., Novak, J., Herr, A.B.: Recognition of galactose-deficient O-glycans in the hinge region of IgA1 by N-acetylgalactosamine-specific snail lectins: A comparative binding study. Biochemistry **49**(27), 5671–5682 (2010). doi:10.1021/bi9019498

69. Suzuki, H., Moldoveanu, Z., Hall, S., Brown, R., Vu, H.L., Novak, L., Julian, B.A., Tomana, M., Wyatt, R.J., Edberg, J.C., Alarcon, G.S., Kimberly, R.P., Tomino, Y., Mestecky, J., Novak, J.: IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. J. Clin. Investig. **118**(2), 629–639 (2008). doi:10.1172/JCI33189
70. Moura, I.C., Arcos-Fajardo, M., Sadaka, C., Leroy, V., Benhamou, M., Novak, J., Vrtovsnik, F., Haddad, E., Chintalacharuvu, K.R., Monteiro, R.C.: Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy. J. Amer. Soc. Nephr. **15**(3), 622–634 (2004)
71. Berthoux, F., Suzuki, H., Thibaudin, L., Yanagawa, H., Maillard, N., Mariat, C., Tomino, Y., Julian, B.A., Novak, J.: Autoantibodies targeting galactose-deficient IgA1 associate with progression of IgA nephropathy. J. Am. Soc. Nephrol. **23**(9), 1579–1587 (2012). doi:10.1681/ASN.2012010053
72. Ruef, C., Budde, K., Lacy, J., Northemann, W., Baumann, M., Sterzel, R.B., Coleman, D.L.: Interleukin 6 is an autocrine growth factor for mesangial cells. Kidney Int. **38**(2), 249–257 (1990)
73. Taniguchi, Y., Yorioka, N., Oda, H., Yamakido, M.: Platelet-derived growth factor, interleukin (IL)-1 beta, IL-6R and tumor necrosis factor-alpha in IgA nephropathy. An immunohistochemical study. Nephron **74**(4), 652–660 (1996)
74. Taniguchi, Y., Yorioka, N., Masaki, T., Asakimori, Y., Yamashita, K., Yamakido, M.: Localization of transforming growth factors beta1 and beta2 and epidermal growth factor in IgA nephropathy. Scand. J. Urol. Nephrol. **33**(4), 243–247 (1999)
75. Grande, J.P.: Role of transforming growth factor-beta in tissue injury and repair. Proc. Soc. Exp. Biol. Med. **214**(1), 27–40 (1997)
76. Yoshioka, K., Takemura, T., Murakami, K., Okada, M., Hino, S., Miyamoto, H., Maki, S.: Transforming growth factor-beta protein and mRNA in glomeruli in normal and diseased human kidneys. Lab. Invest. J. Tech. Methods Pathol. **68**(2), 154–163 (1993)
77. Niemir, Z.I., Stein, H., Noronha, I.L., Kruger, C., Andrassy, K., Ritz, E., Waldherr, R.: PDGF and TGF-beta contribute to the natural course of human IgA glomerulonephritis. Kidney Int. **48**(5), 1530–1541 (1995)
78. Hashimoto, A., Suzuki, Y., Suzuki, H., Ohsawa, I., Brown, R., Hall, S., Tanaka, Y., Novak, J., Ohi, H., Tomino, Y.: Determination of severity of murine IgA nephropathy by glomerular complement activation by aberrantly glycosylated IgA and immune complexes. Am. J. Pathol. **181**(4), 1338–1347 (2012). doi:10.1016/j.ajpath.2012.06.038
79. Tamouza, H., Chemouny, J.M., Raskova Kafkova, L., Berthelot, L., Flamant, M., Demion, M., Mesnard, L., Paubelle, E., Walker, F., Julian, B.A., Tissandie, E., Tiwari, M.K., Camara, N.O., Vrtovsnik, F., Benhamou, M., Novak, J., Monteiro, R.C., Moura, I.C.: The IgA1 immune complex-mediated activation of the MAPK/ERK kinase pathway in mesangial cells is associated with glomerular damage in IgA nephropathy. Kidney Int. **82**(12), 1284–1296 (2012). doi:10.1038/ki.2012.192
80. Gharavi, A.G., Moldoveanu, Z., Wyatt, R.J., Barker, C.V., Woodford, S.Y., Lifton, R.P., Mestecky, J., Novak, J., Julian, B.A.: Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J. Am. Soc. Nephrol. **19**(5), 1008–1014 (2008). doi:10.1681/ASN.2007091052
81. Julian, B.A., Quiggins, P.A., Thompson, J.S., Woodford, S.Y., Gleason, K., Wyatt, R.J.: Familial IgA nephropathy. Evidence of an inherited mechanism of disease. N. Engl. J. Med. **312**(4), 202–208 (1985). doi:10.1056/NEJM198501243120403
82. Beerman, I., Novak, J., Wyatt, R.J., Julian, B.A., Gharavi, A.G.: The genetics of IgA nephropathy. Nature clinical practice. Nephrology **3**(6), 325–338 (2007). doi:10.1038/ncpneph0492
83. Kiryluk, K., Gharavi, A.G., Izzi, C., Scolari, F.: IgA nephropathy–the case for a genetic basis becomes stronger. Nephrology, Dialysis, Transplantation: Official publication of the European Dialysis and Transplant Association - European Renal Association **25**(2), 336–338 (2010). doi:10.1093/ndt/gfp593
84. Lavigne, K.A., Woodford, S.Y., Barker, C.V., Julian, B.A., Novak, J., Moldoveanu, Z., Gharavi, A.G., Wyatt, R.J.: Familial IgA nephropathy in southeastern Kentucky. Clin. Nephrol. **73**(2), 115–121 (2010)
85. Hastings, M.C., Moldoveanu, Z., Julian, B.A., Novak, J., Sanders, J.T., McGlothan, K.R., Gharavi, A.G., Wyatt, R.J.: Galactose-deficient IgA1 in African Americans with IgA nephropathy: Serum levels and heritability. Clin. J. Am. Soc. Nephrol. **5**(11), 2069–2074 (2010). doi:10.2215/CJN.03270410
86. Kiryluk, K., Moldoveanu, Z., Sanders, J.T., Eison, T.M., Suzuki, H., Julian, B.A., Novak, J., Gharavi, A.G., Wyatt, R.J.: Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch-Schonlein purpura nephritis. Kidney Int. **80**(1), 79–87 (2011). doi:10.1038/ki.2011.16
87. Paterson, A.D., Liu, X.Q., Wang, K., Magistroni, R., Song, X., Kappel, J., Klassen, J., Cattran, D., St George-Hyslop, P., Pei, Y.: Genome-wide linkage scan of a large family with IgA nephropathy localizes a novel susceptibility locus to chromosome 2q36. J. Am. Soc. Nephrol. **18**(8), 2408–2415 (2007). doi:10.1681/ASN.2007020241
88. Moldoveanu, Z., Wyatt, R.J., Lee, J.Y., Tomana, M., Julian, B.A., Mestecky, J., Huang, W.Q., Anreddy, S.R., Hall, S., Hastings, M.C., Lau, K.K., Cook, W.J., Novak, J.: Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. Kidney Int. **71**(11), 1148–1154 (2007). doi:10.1038/sj.ki.5002185
89. Ponticelli, C.: Tonsillectomy and IgA nephritis. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association **27**(7), 2610–2613 (2012). doi:10.1093/ndt/gfs093
90. Komatsu, H., Fujimoto, S., Kikuchi, M., Sato, Y., Kitamura, K.: Tonsillectomy delays progression of advanced IgA nephropathy to end-stage kidney disease. Ren. Fail. **34**(4), 448–453 (2012). doi:10.3109/0886022X.2011.653775
91. Maeda, I., Hayashi, T., Sato, K.K., Shibata, M.O., Hamada, M., Kishida, M., Kitabayashi, C., Morikawa, T., Okada, N., Okumura, M., Konishi, M., Konishi, Y., Endo, G., Imanishi, M.: Tonsillectomy has beneficial effects on remission and progression of IgA nephropathy independent of steroid therapy. Nephrology, dialysis, transplantation: Official publication of the European Dialysis and Transplant Association - European Renal Association **27**(7), 2806–2813 (2012). doi:10.1093/ndt/gfs053
92. Goto, T., Bandoh, N., Yoshizaki, T., Nozawa, H., Takahara, M., Ueda, S., Hayashi, T., Harabuchi, Y.: Increase in B-cell-activation factor (BAFF) and IFN-gamma productions by tonsillar mononuclear cells stimulated with deoxycytidyl-deoxyguanosine oligodeoxynucleotides (CpG-ODN) in patients with IgA nephropathy. Clin. Immunol. **126**(3), 260–269 (2008). doi:10.1016/j.clim.2007.11.003
93. Novak, J., Moldoveanu, Z., Julian, B.A., Raska, M., Wyatt, R.J., Suzuki, Y., Tomino, Y., Gharavi, A.G., Mestecky, J., Suzuki, H.: Aberrant glycosylation of IgA1 and anti-glycan antibodies in IgA nephropathy: Role of mucosal immune system. Advances Oto-Rhino-Laryngology **72**, 60–63 (2011). doi:10.1159/000324607
94. Oh, H.J., Ahn, S.V., Yoo, D.E., Kim, S.J., Shin, D.H., Lee, M.J., Kim, H.R., Park, J.T., Yoo, T.H., Kang, S.W., Choi, K.H., Han, S.H.: Clinical outcomes, when matched at presentation, do not vary between adult-onset Henoch-Schonlein purpura nephritis and IgA nephropathy. Kidney Int. **82**(12), 1304–1312 (2012). doi:10.1038/ki.2012.302
95. Dueymes, M., Bendaoud, B., Pennec, Y.L., Youinou, P.: IgA glycosylation abnormalities in the serum of patients with primary Sjogren's syndrome. Clin. Exp. Rheumatol. **13**(2), 247–250 (1995)
96. Xue, J., Zhao, Q., Zhu, L., Zhang, W.: Deglycosylation of FcalphaR at N58 increases its binding to IgA. Glycobiology **20**(7), 905–915 (2010). doi:10.1093/glycob/cwq048