Glycosylation engineering of therapeutic IgG antibodies: challenges for the safety, functionality and efficacy

Yusuke Mimura, Toshihiko Katoh, Radka Saldova, Roisin O’Flaherty, Tomonori Izumi, Yuka Mimura-Kimura, Toshiaki Utsunomiya, Yoichi Mizukami, Kenji Yamamoto, Tsuneo Matsumoto, Pauline M. Rudd

1 Department of Clinical Research, NHO Yamaguchi-Ube Medical Center, 685 Higashi-Kiwa, Ube 755-0241, Japan
2 Laboratory of Molecular Biology and Bioreponse, Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kitashirakawa, Oiwake-Cho, Sakyo-Ku, Kyoto 606-8502, Japan
3 NIBRT GlycoScience Group, National Institute for Bioprocessing Research and Training, Mount Merrion, Blackrock, Dublin 4, Ireland
4 Center for Regenerative Medicine, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami Kogushi, Ube 755-8505, Japan
5 Center for Gene Research, Yamaguchi University, 1-1-1 Minami-Kogushi, Ube 755-8505, Japan
6 Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi, Ishikawa 921-8836, Japan

Correspondence: mimuray66@outlook.jp (Y. Mimura)
Received April 4, 2017 Accepted May 22, 2017

ABSTRACT

Glycosylation of the Fc region of IgG has a profound impact on the safety and clinical efficacy of therapeutic antibodies. While the biantennary complex-type oligosaccharide attached to Asn297 of the Fc is essential for antibody effector functions, fucose and outer-arm sugars attached to the core heptasaccharide that generate structural heterogeneity (glycoforms) exhibit unique biological activities. Hence, efficient and quantitative glycan analysis techniques have been increasingly important for the development and quality control of therapeutic antibodies, and glycan profiles of the Fc are recognized as critical quality attributes. In the past decade our understanding of the influence of glycosylation on the structure/function of IgG-Fc has grown rapidly through X-ray crystallographic and nuclear magnetic resonance studies, which provides possibilities for the design of novel antibody therapeutics. Furthermore, the chemoenzymatic glycoengineering approach using endoglycosidase-based glycosynthases may facilitate the development of homogeneous IgG glycoforms with desirable functionality as next-generation therapeutic antibodies. Thus, the Fc glycans are fertile ground for the improvement of the safety, functionality, and efficacy of therapeutic IgG antibodies in the era of precision medicine.

KEYWORDS chemoenzymatic glycoengineering, crystal structure, endoglycosidase, fucose, glycosylation, intravenous immunoglobulin, sialic acid, transglycosylation, ultra performance liquid chromatography

INTRODUCTION

Glycosylation of proteins is a complex and versatile post-translational modification that influences biological activity, protein conformation, stability, solubility, secretion, pharmacokinetics, and antigenicity (Dwek, 1998). IgG is composed of three globular domain structures, two of which are the fragments for antigen binding (Fab) and the other is the fragment crystallizable (Fc) that activates Fcy receptors (FcyRs) on leukocytes and C1 component of complement. IgG molecules bear oligosaccharides at Asn297 of the Fc region, and the oligosaccharide plays an essential role in Fc effector functions including antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) that are among mechanisms of action of therapeutic antibodies. Therefore, engineering of Fc glycosylation is a
rational strategy to improve the safety and efficacy of therapeutic IgG antibodies. Although the importance of glycosylation for Fc effector functions of therapeutic IgG antibodies has been previously documented (Jefferis, 2009, 2012, 2017; Mimura et al., 2009; Zhang et al., 2016), this review summarizes recent advances in antibody glyobiology that are applicable for optimization of the functionality of IgG antibodies for therapeutic purposes, including the novel glycan profiling technology developed by Rudd’s group (Bones et al., 2010), the influence of glycosylation on the structure and function of the Fc revealed from crystal structures of nonglycosylated Fc, the nonfucosylated Fc-glycosylated Fc receptor IIIa (FcγRIIa) complexes and sialylated Fc and a new approach to engineering of IgG glycoforms via transglycosylation of predefined oligosaccharides to deglycosylated IgG-Fc.

STRUCTURE OF THE IGG-FC GLYCANS

The IgG-Fc glycans released from human normal polyclonal IgG are highly heterogeneous (Arnold et al., 2006; Mimura et al., 2009; Rudd and Dwek, 1997) (Fig. 1A), due to the variable addition of fucose, bisecting GlcNAc, galactose, and sialic acid residues to the core complex biantennary heptasaccharide (GlcNAc₂Man₇GlcNAc₂, designated G0). The heterogeneous glycans can be classified into three sets (G0, G1, and G2), depending on the number of galactose residues in the outer arms of biantennary glycans. Within each of these sets are four species that result from the presence or absence of core fucose and bisecting GlcNAc, namely, 16 neutral complex-type structures. Figure 1A shows the glycan profile of intravenous immunoglobulin (IVIG, Kenketsu Venilon-I, Teijin Phama) which is a therapeutic preparation of healthy donors. The fluorescein-labeled glycans from the Fc fragment of IVIG (IVIG-Fc) were separated into >20 peaks by hydrophilic interaction liquid chromatography (HILIC) in which fucosylated, monogalactosylated (G1F) glycoforms predominate, with a preference for galactosylation on the α(1-6)-arm (G1[6F]) over the α(1-3)-arm (Fig. 1A-i, Table 1) (Pucic et al., 2011). The proportion of the sialylated glycoforms of IVIG-Fc was approximately 19% (Table 1), and sialic acid is known to be added preferentially on the α(1-3)-arm of the digalactosylated (G2) glycoforms (Barb et al., 2009; Grey et al., 1982; van den Eijnden et al., 1980). Sialylation occurs in α(2-6)-linkage with N-acetyllactosamine (NeuAc) in humans whereas it is in α(2-3)-linkage in Chinese hamster ovary (CHO)-derived recombinant IgG molecules (Takeuchi et al., 1988). The glycan profile of Sambucus nigra agglutinin (SNA)-bound IVIG-Fc showed the prominent peaks of monosialylated and disialylated glycans with and without bisecting GlcNAc (>60%, Fig. 1A-ii, Table 1). The presence of around 40% of nonsialylated glycans released from the SNA-bound Fc indicates that sialylation of one of the two Fc glycans is sufficient for the Fc to bind to SNA. The glycans of the therapeutic monoclonal IgG antibodies nivolumab (Opdivo®, Ono Pharmaceutical), bevacizumab (Avastin®, Chugai Pharmaceutical), and mogamulizumab (Poteligeo®, Kyowa Hakko Kirin) were less heterogeneous than those of IVIG-Fc (Fig. 1B-D). Currently approved therapeutic IgG antibodies are produced in CHO, NS0 and Sp2/0 cell lines, and nivolumab and bevacizumab are produced in CHO cells (Fig. 1B and C) and mogamulizumab in α(1-6)-fucosyltransferase (FUT8)-deficient CHO cells (Fig. 1D), which clearly shows the presence and absence of core fucose, respectively. Non-galactosylated glycoforms (G0F and G0) predominated, and sialylated glycans were negligible for these CHO-derived IgG antibodies (Table 1). Recombinant IgG antibodies produced from CHO and murine cells do not contain bisecting GlcNAc in contrast to human IgG as observed for IVIG (Fig. 1A) (Raju et al., 2000).

Terminal α(1-3)-linked galactose (α(1-3)-Gal) and N-glycolyneuraminic acid (NeuGc) residues are frequently found in the N-glycans of recombinant IgG antibodies produced from murine myeloma cells. Such glycan structures are unnatural and potentially immunogenic in humans. The α-galactosylation and sialylation with NeuGc are reported in cetuximab produced from Sp2/0 (Qian et al., 2007) and infliximab from NS0 (Mimura et al., 2009) and are markedly increased for an IgG1-F243A mutant when expressed in murine cells (Mimura et al., 2016). It has been reported that all humans have IgG antibodies specific for the α(1-3)-Gal epitope (Gallili et al., 1993) and that the anti-NeuGc activity is
Glycoengineering for next-generation therapeutic antibodies

A. IVIG-Fc

(i) Control

+ sialidase

(ii) SNA-bound IVIG-Fc

+ sialidase

B. Nivolumab

C. Bevacizumab

D. Mogamulizumab

© The Author(s) 2017. This article is an open access publication
The IgG-Fc crystal structure reveals the oligosaccharide as integral to the Fc structure, sequestered in the internal space enclosed by the two C1 domain (Fig. 2). Glycans shown as green sticks. The electron density map provides coherent diffraction for the monogalactosylated oligosaccharide and allows the possibility of >70 contacts with 14 amino acid residues of the C1 domain (Deisenhofer, 1981; Padlan, 1990). The crystal structure of the complex between IgG1-Fc and an E. coli-produced soluble recombinant form of FcγRIIa (sFcγRIIa) has demonstrated that the FcγRII binds to the lower hinge and the hinge proximal regions of the two C1 domains asymmetrically with a 1:1 stoichiometry (Radaev et al., 2001; Sondermann et al., 2000). In the complex of the Fc with the aglycosylated sFcγRIIa, the Fc glycans are not directly associated with sFcγRIIa except the primary GlcNAc of one oligosaccharide although removal of the Fc glycans abrogates sFcγRIIa binding. Importantly, the interaction between IgG-Fc and sFcγRIIa can be substantially influenced by the presence or absence of fucosylation of the Fc and glycosylation of sFcγRIIa (see below) (Ferrara et al., 2011; Mizushima et al., 2011). Notably, the horseshoe-shaped Fc opens up upon complex formation, and therefore it is presumed that the Fc glycan maintains the open conformation of the Fc and that removal of the Fc glycan results in a closed conformation. This notion is supported by the crystal structures of the Fc glycoforms bearing sequentially truncated glycans ((G2F)2, (G0F)2, (M3N2F)2 and (MN2F)2, G: galactose; M: mannose; N: GlcNAc; F: fucose) in which the (G2F)2 glycoform shows the longest Pro329-Pro329 Cα distance of 33.7 Å whereas the (MN2F)2 glycoform the shortest distance of 21.9 Å (Krapp et al., 2003). Although truncation of the terminal sugar residues results in an increase of destabilization of the C1 domain and a reduction of affinity to sFcγRIIa (Mimura et al., 2001) and sFcγRIIa (Yamaguchi et al., 2006), the profound influence of Fc glycosylation on FcγR binding has not been paralleled by gross conformational differences between glycosylated and aglycosylated Fc fragments (Lund et al., 1990).

**Influence of the Fc Glycan on Antibody Effector Functions**

The oligosaccharides at Asn297 of the IgG-Fc are essential for the optimal activation of FcγRs and complement C1 although the carbohydrate moiety accounts for only 2%–3% of the IgG molecule. The clearance mechanisms including phagocytosis, ADCC, and CDC mediated by Fcγ receptors and C1q are abrogated or severely compromised for aglycosylated or deglycosylated forms of IgG (Nose and Wigzell, 1983; Pound et al., 1993; Sarmay et al., 1992; Tao and Morrison, 1989; Woof and Burton, 2004). The IgG-Fc crystal structure demonstrates that the Fc glycan maintains the open conformation of the Fc, and removal of the Fc glycan results in a closed conformation (Radaev et al., 2001; Padlan, 1990). The crystal structure of the complex between IgG1-Fc and an E. coli-produced soluble recombinant form of FcγRIIa (sFcγRIIa) has demonstrated that the FcγRII binds to the lower hinge and the hinge proximal regions of the two C1 domains asymmetrically with a 1:1 stoichiometry (Radaev et al., 2001; Sondermann et al., 2000). In the complex of the Fc with the aglycosylated sFcγRIIa, the Fc glycans are not directly associated with sFcγRIIa except the primary GlcNAc of one oligosaccharide although removal of the Fc glycans abrogates sFcγRIIa binding. Importantly, the interaction between IgG-Fc and sFcγRIIa can be substantially influenced by the presence or absence of fucosylation of the Fc and glycosylation of sFcγRIIa (see below) (Ferrara et al., 2011; Mizushima et al., 2011). Notably, the horseshoe-shaped Fc opens up upon complex formation, and therefore it is presumed that the Fc glycan maintains the open conformation of the Fc and that removal of the Fc glycan results in a closed conformation. This notion is supported by the crystal structures of the Fc glycoforms bearing sequentially truncated glycans ((G2F)2, (G0F)2, (M3N2F)2 and (MN2F)2, G: galactose; M: mannose; N: GlcNAc; F: fucose) in which the (G2F)2 glycoform shows the longest Pro329-Pro329 Cα distance of 33.7 Å whereas the (MN2F)2 glycoform the shortest distance of 21.9 Å (Krapp et al., 2003). Although truncation of the terminal sugar residues results in an increase of destabilization of the C1 domain and a reduction of affinity to sFcγRIIa (Mimura et al., 2001) and sFcγRIIa (Yamaguchi et al., 2006), the profound influence of Fc glycosylation on FcγR binding has not been paralleled by gross conformational differences between glycosylated and aglycosylated Fc fragments (Lund et al., 1990).

**Structures of Nonglycosylated IgG-Fc**

Recently crystal structures have been solved for E. coli-expressed, aglycosylated murine IgG1-Fc (PDB ID code: 3HKF) and glycosylated human IgG1-Fc (PDB ID code: 3S7G) (Fig. 2A) (Borrok et al., 2012) and enzymatically deglycosylated human IgG1-Fc (PDB ID code: 3DNK) (Fig. 2B-i) and deglycosylated IgG4-Fc (PDB ID code: 4D2N) (Fig. 2B-ii) (Davies et al., 2014a). The bacterially expressed murine aglycosylated IgG1-Fc shows a strongly closed conformation (Feige et al., 2009). The crystal structure of aglycosylated human IgG1-Fc (PDB ID code: 3S7G) reveals two Fc dimers of the asymmetric unit, interfacing at the C1-C2 elbow between the dimers, and adopts a closed Fc conformation with Pro329-Pro329 Cα distances of 18.9 Å and 19.6 Å for the two Fc molecules whereas the structure of native Fc (PDB ID code: 3AVE) shows the Pro329-Pro329 distance of 25.1 Å (Fig. 2A) (Borrok et al., 2012). Furthermore, significant disorder is observed in the C′E loop (Gln293–Phe303) that contains the N-glycosylation site and

| Table 1. Analysis of the key features of the N-glycans released from the therapeutic IgG antibodiesa |
|-----------------------------------------------|
| IgG                                          |
| Sialylation (%)                              |
| Term. Gal (%)b                               |
| Term. GlcNAc (%)                             |
| Bisecting GlcNAc (%)                         |
| Core fucose (%)c                            |
| Predominant glycoform                       |
| S1   | S2   | (%)  | (%)   | (%)  | (%)   | (%)  |         |
| IVIG-Fc | 16.2 | 2.6  | 62   | 19.2 | 11.4 | 92.8 | G1F     |
| SNA-IVIG-Fc | 32.5 | 30.2 | 27.2 | 10.1 | 36.1 | 89.4 | G2FBS1  |
| Nivolumab | 1.3  | 0    | 42.4 | 56.3 | 0    | 100  | G0F     |
| Bevacizumab | 0    | 0    | 19   | 81   | 0    | 97.4 | G0F     |
| Mogamulizumab | 0    | 0    | 36.3 | 63.7 | 0    | 0    | G0      |

a Glycans were quantitated by measuring peak areas in the HILIC profiles (Fig. 1).
b Glycoforms originating in galactose residues (G1, G1F, G1FB, G2, G2F, G2FB).
c Glycoforms originating in GlcNAc residues (G0 and G0F).
a region crucial for FcγR binding. In contrast, the crystal structure of enzymatically deglycosylated human IgG1-Fc (PDB ID code: 3DNK) reveals an open conformation (Pro329–Pro329 distance, 27.6 Å) (Fig. 2B-i). The crystal structure of the enzymatically deglycosylated human IgG4-Fc myeloma protein (Rea) reveals two interlocked Fc molecules with the Cγ2 domains oriented in a symmetric open conformation (Pro329–Pro329 distance, 29.1 Å) (Fig. 2B-ii) (Davies et al., 2014a). There are no significant differences between the overall structures of deglycosylated IgG4-Fc and glycosylated IgG4-Fc although the conformation of the CE loop is altered in the absence of the oligosaccharide (Davies et al., 2014a; Davies et al., 2014b). Thus, it seems likely that nonglycosylated Cγ2 domains can adopt not only closed but also flexible orientations. Furthermore, the aglycosylated human IgG1-Fc in Fig. 2A displays larger radii of gyration than glycosylated Fc by small angle X-ray scattering, which suggests a more open Cγ2 domain conformation in solution (Borrok et al., 2012).

![Figure 2. Comparison of nonglycosylated and glycosylated Fc structures.](image)

**AGLYCOSYLATED IGG ANTIBODIES FOR THERAPY**

Aglycosylated antibodies are suited for purposes where ADCC/CDC action is not required as is the case for neutralizing, agonistic or antagonistic antibodies. Numerous aglycosylated IgG antibodies are under clinical evaluation including otelixizumab (TRX4), onartuzumab (MetMab), and clazakizumab (ALD518) (Ju and Jung, 2014; Jung et al., 2011). The use of aglycosylated IgG antibodies provides the following advantages: (1) The serum half-life of aglycosylated IgG is shown to be comparable to that of glycosylated counterpart in chimpanzees (Simmons et al., 2002); (2) Aglycosylated IgG can be produced in lower eukaryotes or in bacteria, which provides bioprocessing advantages in terms of shorter bioprocess development and running times without need to consider glycan heterogeneity problems; (3) Aglycosylated IgG antibodies maintain the ability to engage some of the FcγRs by a small subset of substitutions in the Cγ2 and/or Cγ3 domains. An aglycosylated IgG variant with S298G/T299A mutations has been identified that activates FcγRIIa (Sazinsky et al., 2008). In addition, an aglycosylated IgG variant with mutations E382V/M428I within the Cγ3 domain has been shown to mediate cytotoxicity of target cells via FcγRI (Ju et al., 2015; Jung et al., 2010). Thus, engineering of aglycosylated IgG provides new routes for the design of therapeutic antibodies with customized functionality.

Deglycosylation of circulating IgG in vivo by administration of endoglycosidase from *Streptococcus pyogenes* (Endo-S) has been considered as a novel therapeutic strategy for immune evasion in patient with autoimmune disorders (Allhorn and Collin, 2009; Collin et al., 2008). Endo-S selectively hydrolyzes the glycosidic bond of the chitobiose core of the Fc glycans leaving the primary GlcNAc with or without fucose, and Endo-S treatment of IgG results in a severely reduced affinity to FcγRs. Administration of recombinant Endo-S to mice has been shown to transiently remove the Fc glycans from circulating IgG and suppress inflammation in autoimmune models including immune thrombocytopenic purpura (ITP) and serum transfer arthritis (Albert et al., 2008). As a novel approach to enhance the efficacy of therapeutic antibodies, both Endo-S and therapeutic IgGs bearing Endo-S-resistant high mannose-type glycans are administered to eliminate competition for FcγR binding between circulating IgG and therapeutic IgG so that the therapeutic IgG could efficiently exert effector functions (Baruah et al., 2012). However, repeated administrations of the bacteria-derived endoglycosidase may lead to the
development of antibodies against the enzyme. It should also be noted that immune complexes formed with Endo-S-treated IgG retain the ability to activate FcγRs in an IgG subclass-dependent manner. Human IgG1 and IgG3 antibodies deglycosylated by Endo-S are found to be able to activate FcγRs (Kao et al., 2015). Thus, the therapeutic efficacy of the Endo-S administration may not be predictable where FcγR activation via multivalent immune complexes is involved in disease pathogenesis. The remarkable specificity of Endo-S for native IgG has also been exploited for engineering of IgG glycoforms as described below.

**INFLUENCE OF FC GLYCAN STRUCTURE ON PHARMACOKINETICS OF IGG ANTIBODIES**

Clearance has a critical impact on the efficacy of therapeutic antibodies. IgG antibodies are protected from rapid degradation in lysosomes through the neonatal Fc receptor (FcRn) recycling mechanism, which explains the long half-life of IgG antibodies in the serum (Roopenian and Akiles, 2007). FcRn interacts with IgG at the C12/C13 interface, independently of the Fc glycan. Other receptors that are known to bind and clear proteins with specific glycans include the asialoglycoprotein receptor that binds to terminal galactose residues of N-glycans (Ashwell and Harford, 1982) and the mannose receptor that recognizes terminal mannose or GlcNAc sugars (Lee et al., 2002). High mannose glycoforms are frequently found in recombinant IgG antibodies produced from tissue culture CHO and murine cells (Goetze et al., 2011; Mimura et al., 2009; Zhang et al., 2016). Shorter half-lives have been demonstrated for IgG antibodies bearing high mannose-type glycans compared with those with the complex-type glycans in mice (Kanda et al., 2007) and human FcRn-transgenic mice (Liu et al., 2011). When therapeutic IgG1 or IgG2 antibody was administered in human subjects, the relative abundance of IgG glycoforms with terminal galactose or GlcNAc remained constant during 34 days after injection while high mannose glycoforms were selectively cleared more rapidly at lower intravenous doses (Goetze et al., 2011). Thus, the presence of high mannose glycoforms may compromise the efficacy of antibody therapeutics through enhanced clearance and/or possible immunogenicity elicited by uptake of immune complexes via the mannose receptor on macrophages/dendritic cells and the activation of the mann-binding lectin pathway (Arnold et al., 2006; Jefferis, 2017). The Fab is also glycosylated in approximately 20% of polyclonal human IgG, and the Fab glycans can be of highly galactosylated and sialylated complex-type (Holland et al., 2006; Mimura et al., 2007) or of high mannose-type, depending on the location of the glycosylation site in the Vκ region (Gala and Morrison, 2004; Radcliffe et al., 2007; Wright et al., 1991). As Fab glycosylation can modulate the antibody binding (Wright et al., 1991) and physicochemical properties (Wu et al., 2010) and in vivo clearance as observed for highly glycosylated Fc-fusion proteins (Higel et al., 2016; Liu, 2015, 2017), the variable region glycosylation may also be exploited to improve the efficacy of antibody therapeutics.

**BIOLOGICAL ACTIVITY OF CORE FUCOSE RESIDUE**

The impact of fucose depletion from the IgG-Fc glycan on ADCC probably represents one of the most important discoveries in antibody glyobiology. The dramatic enhancement of ADCC is attributed to the improved affinity of nonfucosylated IgG for FcγRIIa expressed on natural killer (NK) cells (Kanda et al., 2007; Okazaki et al., 2004; Shields et al., 2002; Shinkawa et al., 2003; Yamane-Ohnuki et al., 2004). In the past century, the biological relevance of core fucosylation received relatively little attention, in part, due to difficulty in the removal of core fucose from the IgG-Fc. Although the influence of the fucose residue on the stability of the Fc was examined by differential scanning calorimetry, fucosylated and nonfucosylated human IgG1-Fc proteins did not show any significant difference in the stability (Mimura et al., 2000; Mimura et al., 2001). However, the discovery of the importance of fucose depletion needed an appropriate binding partner, i.e., glycosylated (mammalian cell-expressed) FcγRIIa. On the other hand, preparation of biologically active aglycosylated (E. coli-expressed) FcγRs was optimized in the late 1990s (Sondermann and Jacob, 1999), which led to the first crystallographic analyses of FcγRIIb (Sondermann et al., 1999) and the Fc–sFcγRIIa complex (Sondermann et al., 2000). When the binding of fucosylated Fc to aglycosylated FcγRIIa was analyzed by surface plasmon resonance (Maenaka et al., 2001), the Fc affinity to aglycosylated FcγRIIa was slightly higher than that to glycosylated (CHO cell-expressed) FcγRIIa observed by analytical ultracentrifugation (Ghirlando et al., 1995). Therefore, it was presumed at this time that N-glycosylation of FcγRII negatively influences the Fc–FcγRIIa interaction. Rather than core fucose, bisecting GlcNAc drew attention because recombinant IgG1 bearing bisected glycans by overexpression of β1,4-N-acetylgalcosaminyltransferase III (GnT-III) exhibited improved ADCC (Umana et al., 1999), which later proved to be due to inhibition of α(1-6)-fucosyltransferase (FUT8) by the presence of bisecting GlcNAc.

Biological relevance of core fucose in the Fc glycan was demonstrated by two groups (Shields et al., 2002; Shinkawa et al., 2003). The Genentech group expressed glycosylated FcγRI, FcγRIIa, FcγRIII and FcγRIIa in mammalian cell lines (Shields et al., 2001) and humanized anti-HER2 and anti-IgE IgG1 antibodies with low fucose contents (ca. 10% and 21%, respectively) in Lec13 cells, a variant CHO cell line deficient in fucosylation (Shields et al., 2002). Lack of core fucose in the Fc glycan enhanced the binding of IgG to FcγRIIa up to 50-fold, together with slightly improved binding to the Arg131 FcγRIIa polymorphic form and FcγRIIb
whereas the absence of fucose did not affect the binding to human FcγRI, C1q, and neonatal FcR. The BioWa (or Kyowa Hakko Kirin) group produced an anti-CD20 antibody with low fucose contents (5%) in rat YB2/0 B-lymphoblast cells, and the antibody exhibited enhanced ADCC using human peripheral blood mononuclear cells (Shinkawa et al., 2003). The latter group has also found that increased bisecting GlcNAc contents in the nonfucosylated IgG glycan fractionated by PHA-E lectin affinity chromatography do not show additive effect on ADCC, which suggests that it is not bisecting GlcNAc but core fucose that markedly influences ADCC. The study also demonstrated the low expression level of FUT8 mRNA in the YB2/0 B-cells, which led to the establishment of FUT8 double gene-knockout CHO/DG44 cell line for production of completely nonfucosylated antibodies (Yamane-Ohnuki et al., 2004). It is known that human IgG1 binds more strongly to NK cells expressing homozygous FcγRIIa-Val158 than to those expressing FcγRIIa-Phe158 (Koene et al., 1997; Wu et al., 1997). IgG devoid of core fucose showed improved binding to both FcγRIIa-Val158 and FcγRIIa-Phe158, with the affinity being increased up to 50-fold and 30-fold, respectively (Ferrara et al., 2006; Shields et al., 2002). Importantly, the glycan at Asn162 of FcγRIIa is crucial for the high affinity of nonfucosylated IgG to FcγRIIa whereas the glycan at Asn45 is required for proper folding but has a negative effect on the binding (Shibata-Koyama et al., 2009). X-ray crystallographic analysis of nonfucosylated Fc fragments produced in the FUT8−/− CHO/DG44 cells revealed a similar structure to the fucosylated counterpart (pdb id codes: 2DTQ and 2DTS), with subtle difference in conformation around Tyr296 near the fucose residue. The stable-isotope-assisted NMR analyses also confirmed the similarity of the overall structures in solution (Matsumiya et al., 2007).

Crystal structures of the complex between nonfucosylated Fc and glycosylated FcyRIIa have been solved by two independent groups (Ferrara et al., 2011; Mizushima et al., 2011). The crystal structure of the complex from Ferrara et al. was obtained with human nonfucosylated IgG1 produced from CHO-K1SV cells that overexpressed GnT-III to block the action of FUT8 and the sFcyRIIa variant glycosylated at Asn45/Asn162 produced in HEK293-EBNA cells treated with the mannosidase I inhibitor, kifunensine (pdb id code: 3SGK). The sFcyRIIa bearing oligomannose glycans at the two sites binds nonfucosylated IgG1 with comparable affinity to the fully glycosylated FcyRIIa. The crystal structure reveals unique interactions between the nonfucosylated glycan of the Fc and the high mannose-type glycan at Asn162 of sFcyRIIa. The absence of core fucose allows hydrogen bonding between the chitobiase core of the glycan at Asn162 of sFcyRIIa and the primary GlcNAc of the Fc glycan of the chain A (Fig. 3A). The terminal mannose residue on the α(1-3)-arm of the high mannose-type glycan of sFcyRIIa forms a hydrogen bond to the Gln295 residue of the Fc. In addition, Tyr296 of the Fc makes contacts between the branching β-mannose and Lys128 residue of sFcyRIIa (Fig. 3A). On the other hand, the crystal structure of the fucosylated Fc–glycosylated sFcyRIIa complex (pdb id code: 3SGJ) reveals that core fucose inhibits the ligand–receptor binding, due to steric hindrance (Fig. 3B). The other crystal structure of the nonfucosylated Fc–FcyRIIa complex from Mizushima et al. was obtained with nonfucosylated IgG from the FUT8−/− CHO cells (Ms704) and sFcyRIIa glycosylated at Asn45/Asn162 from CHO/DG44 cells (pdb id code: 3AY4) (Mizushima et al., 2011). As revealed in the former crystal structure, the binding is mediated by the carbohydrate–carbohydrate and carbohydrate–protein interactions although this sFcyRIIa bears biantennary fucosylated complex-type glycans, in contrast to high mannose-type glycans for the former. The glycan at Asn162 of sFcyRIIa interacts with the nonfucosylated glycan and the Tyr296 and Arg301 residues of the Fc, thereby stabilizing the complex formation. These crystal structures demonstrate that the ADCC activity of nonfucosylated IgG is enhanced by the carbohydrate–carbohydrate interactions through van der Waals force, hydrogen bonding, and hydrophobic interactions. This is a novel mode of the ligand–receptor binding that provides an opportunity to explore optimal combinations of glycoforms of a ligand with those of a receptor to design glycosylated biological therapeutics.

Several nonfucosylated IgG antibodies have already entered the clinic. The phase I clinical trial of nonfucosylated humanized anti-CC chemokine receptor 4 (CCR4) IgG1 antibody KW-0761 (mogamulizumab, Fig. 1D) was initiated in patients with relapsed adult T cell leukemia or peripheral T-cell lymphoma in 2006 (Yamamoto et al., 2010). The phase II clinical trial demonstrated potent antitumor activity and tolerable toxicity profile by mogamulizumab monotherapy (Ishida et al., 2012), which led to approval by the regulatory authority in Japan in 2012. Several other nonfucosylated IgG antibodies are under clinical evaluation, including the ones against OX40 (KHK4083), IL-6R (benralizumab) (Wang et al., 2017), EGFR (immatuzumab) (Delord et al., 2014), and CD20 (obinutuzumab). Fucose depletion of existing antitumor therapeutic IgG antibodies such as rituximab and trastuzumab has been shown to enhance the ADCC activities ex vivo (Iida et al., 2006; Mossner et al., 2010). The anti-CD20 humanized IgG1 antibody obinutuzumab produced in CHO-K1 cells engineered to overexpress GnT-III and Golgi β-mannosidase II exhibits low fucose contents in the Fc glycans and superior antitumor activities to rituximab (Sehn et al., 2012; Sehn et al., 2015). Obinutuzumab was approved in the United States in 2013 for treatment of follicular lymphoma. Thus, nonfucosylated IgG antibodies will be further developed as next-generation therapeutic antibodies with potent ADCC at reduced doses.

**BIOLoGICAL ACTIVITY OF THE TERMINAL SIALIC ACID RESIDUES**

Influence of sialylation on the structure of the Fc has been analyzed by NMR and X-ray crystallographic analysis (Ahmed et al., 2014; Barb et al., 2009; Barb et al., 2012;
By NMR spectroscopy, the relaxation rates of the galactose resonances for the Fc monosialylated on the α(1-3)-arm and the disialylated Fc were found to be largely similar to those for the G2F Fc glycoform, which suggests that Fc sialylation has a minor effect on the motional behavior of the N-glycan. Although the sialic acid residues are highly dynamic and free of strong interaction with the protein moiety of the Fc, the sialylated glycan–Cγ1 polypeptide interactions are largely mediated by the carbohydrate residues up to galactose (Barb et al., 2012). The crystal structure of sialylated Fc (PDB ID code: 4BYH) provides consistent findings with the solution-state NMR measurements (Fig. 4A). The terminal sialic acid on the α(1-6)-arm (Fig. 4A, shown in red) projects away from the protein surface in a solvent-exposed manner, and the monosaccharides on the α(1-3)-arm are visible up to GlcNAc for the Fc chains (Crispin et al., 2013). The lack of electron density for terminal sialic acid residues on the α(1-3)-arm is consistent with the dynamics of the terminal sialic acid observed by the NMR study. This crystal structure of the enzymatically sialylated Fc does not show gross conformational change as compared with that of the native Fc (PDB ID code: 1H3Y, Fig. 4A) whereas those of the disialylated Fc fragments prepared by chemoenzymatic glycoengineering show both
open and closed C12 domain conformations in the crystal (Fig. 4B, PDB ID code: 4Q6Y) (Ahmed et al., 2014). The distances between the Cα atoms at the Pro238 residues of the C12 domains for the open and closed conformers of the disialylated Fc and a representative native Fc (PDB ID code: 3AVE) are 13 Å, 20.2 Å and 19.3 Å, respectively. Although it is unclear whether the conformational heterogeneity of the disialylated Fc results from sialylation or crystal packing contacts, increased conformational flexibility of the sialylated Fc may be associated with anti-inflammatory properties of this glycoform as described below (Ahmed et al., 2014).

Sialylated glycans of the Fc have recently drawn increased attention as an active component of IVIG that exerts anti-inflammatory properties. IVIG has been used to treat not only immunodeficiency (hypogammaglobulinemia) but also various autoimmune diseases including idiopathic thrombocytopenic purpura and Kawasaki disease. It has been shown that the anti-inflammatory effects of IVIG reside in the Fc region of IVIG and that infusion of Fc fragments ameliorates the conditions of children with acute immune thrombocytopenic purpura (Debre et al., 1993). It has been proposed that IVIG binds to an inhibitory FcyRIIB that transmits an inhibitory signal in the cytoplasm to suppress inflammation. The protective effect of IVIG was associated with the induced expression of an inhibitory Fc receptor FcyRIIB (Bruhns et al., 2003) although a correlation between the sialylation level and the anti-inflammatory activity of IgG was not provided.

Multiple effects of Fc sialylation on antibody effector functions and the immune system have been reported including reduction of ADCC (Kaneko et al., 2006; Scallon et al., 2007) and CDC (Quast et al., 2015) and induction of TNF cytokine IL-33 and upregulation of FcyRIIB (Anthony et al., 2011). Sialylated forms of IgG enriched with Sambucus nigra agglutinin (SNA) show reduced affinity to FcyRIIB, thereby reducing the ability of the IgG autoantibodies to trigger in vivo cytotoxicity (Kaneko et al., 2006). In addition to this inhibitory effect, SNA-enriched IVIG-Fc is shown to exert anti-inflammatory activity in the murine K/BxN serum transfer arthritis model equivalent to unfractionated IVIG-Fc at a 10-fold lower dose. The anti-inflammatory activity of SNA-enriched IVIG has been recapitulated with highly sialylated, recombinant human IgG1-Fc (Anthony et al., 2008a). The receptor required for the anti-inflammatory effect of the sialylated IgG has been identified as the C-type lectin, SIGN-R1, expressed on murine splenic macrophage (Anthony et al., 2008b), and its human orthologue DC-SIGN has been shown to act as a receptor for sialylated IgG in human DC-SIGN transgenic mice (Anthony et al., 2011). The proposed mechanism by which sialylated IgG exerts anti-inflammatory effects is TNF cytokine IL-33 expression in SIGN-R1+ or DC-SIGN+ macrophages/dendritic cells through interaction with sialylated IgG. IL-33 then suppresses inflammation by induction of IL-4 from basophils, which leads to upregulation of inhibitory receptor FcyRIIB on effector macrophages. However, the anti-inflammatory activity of sialylated IgG has not been reproduced in some mouse models of autoimmune diseases. No differences were observed between SNA-enriched IVIG and neuraminidase-treated IVIG in the efficacy to ameliorate ITP (Guhr et al., 2011; Leontyev et al., 2012b), K/BxN serum transfer arthritis (Campbell et al., 2014), and experimental autoimmune encephalomyelitis (Othy et al., 2014). In the K/BxN serum arthritis model, depletion of basophils did not influence the anti-inflammatory effect of IVIG. The requirement of FcyRIIB for anti-inflammatory effects of IVIG was not demonstrated by using FcyRIIB-knockout mice (Bazin et al., 2006; Leontyev et al., 2012a). Furthermore, neither sialylated nor native Fc was shown to bind to recombinant DC-SIGN although the binding of serum IgG and its deglycosylated, desialylated, and sialylated glycoforms to DC-SIGN was comparable, indicating that the DC-SIGN binding to IgG could be attributed to cross-reactive, polyclonal Fab specificities (Yu et al., 2013). It has been shown that F(ab′)2 fragments of IVIG could directly interact with DC-SIGN on dendritic cells, which ultimately leads to expansion of Treg cell populations (Trinath et al., 2013). It seems that the discrepancies of the anti-inflammatory effects
of sialylated IgG among these studies have arisen due to different IVIG-Fc preparations at differing sialylation levels in the presence or absence of F(ab′)2, different glycan analysis methods and different genetic backgrounds of mice. Another key question exists around the anti-inflammatory properties of IVIG regarding the identification of the human counterpart of the DC-SIGN+ macrophage from the DC-SIGN-transgenic mouse. Thus, the impact of sialylation of IgG-Fc on immunosuppression in autoimmune diseases remains unsolved, and further studies are needed to elucidate the mechanism of action of IVIG.

CHEMOENZYMATIC GLYCOENGINEERING

Separation of various glycoforms to investigate the biological relevance of glycosylation is a real challenge with glycoproteins. Although cell engineering through overexpression or disruption of relevant enzyme genes have been employed to produce specific glycoforms of IgG (Ha et al., 2011; Li et al., 2006; Raymond et al., 2015; Umana et al., 1999; Yamane-Ohnuki et al., 2004), it is still challenging to optimize the production of desired glycoforms of IgG. Recently, transglycosylation reactions have been applied to synthesis of new glycoconjugates that consist of deglycosylation by an endo-β-N-acetylgalactosaminidase (ENGase) to leave the innermost GlcNAc with or without core fucose at the N-glycosylation site(s) and subsequent reglycosylation by an ENGase-based glycosynthase to transfer a predefined N-glycan substrate to the innermost GlcNAc (Giddens and Wang, 2015; Huang et al., 2012; Umekawa et al., 2010) (Fig. 5). This technique utilizes highly active glycan oxazolines, the mimics of the transition state, as donor substrates (Kobayashi et al., 1996), and transglycosylation with the synthetic glycan oxazoline proceeds in both a stereo- and regiосспhesive manner (Li et al., 2005). This chemoenzymatic glycoengineering is recognized as one of the most promising approaches to synthesize homogeneous glycoforms of a given glycoprotein including IgG and has been applied to the synthesis of fully sialylated IgG glycoforms which would otherwise be quite difficult (Ahmed et al., 2014; Kurogochi et al., 2015; Lin et al., 2015).

Several ENGases possess transglycosylation activity, including Endo-A from Arthrobacter prothormiae (Takegawa et al., 1995; Takegawa et al., 1997), Endo-M from Mucor hiemalis (Fujita et al., 2004; Yamamoto et al., 1994), Endo-D from Streptococcus pneumoniae (Fan et al., 2012; Muramatsu et al., 2001), and Endo-CE from Caenorhabditis elegans (Kato et al., 2002) in the glycoside hydrolase (GH)-85 family and Endo-S from Streptococcus pyogenes (Huang et al., 2012) and Endo-S2 from Streptococcus pyogenes of serotype M49 (Li et al., 2016) in the GH18 family. Various glycosynthase mutants of ENGases have been generated to abolish the hydrolytic activity on the transglycosylation products and improve the transglycosylation efficiency, including Endo-A-N171A, Endo-M-N175Q (Fig. 5B), Endo-S-D233Q, and Endo-S2-D184M. Different ENGases have distinct substrate specificity and limitations. Endo-M acts on both the complex-type and high mannose-type oligosaccharides whereas Endo-A and Endo-S are limited to action on the high mannose-type and the complex-type.

Figure 5. Glycosylation remodeling of IgG using Endo-S and Endo-M-N175Q. (A) Schematic representation of the chemoenzymatic glycoengineering method. SG-Ox, sialoglycan-oxazoline. (B) SDS-PAGE of the transglycosylation product of IgG. Mogamulizumab (1 mg) was deglycosylated with Endo-S (2,000 U, New England Biolabs) in 50 mmol/L acetate containing 5 mmol/L CaCl2 (pH 5.5) and purified on a protein G column. The deglycosylated IgG (0.5 mg) was incubated with Endo-M-N175Q (100 mU, Tokyo Chemical Industry, Japan) and 1 mg of SG-Ox (Fushimi Pharmaceutical, Japan) in 50 μL of 50 mmol/L sodium phosphate (pH 6.5) at 30°C for 2 h. CBB, Coomassie brilliant blue; SNA, Sambucus nigra agglutinin.
respectively. In addition, Endo-S and Endo-S2 in GH18 act on both nonfucosylated and fucosylated glycans whereas ENGases in GH85 are generally inactive on fucosylated glycans except Endo-D. The substrate specificity of an ENGase for transglycosylation is not always the same as that of the wildtype ENGase for hydrolysis, e.g., Endo-D prefers fucosylated glycans for hydrolysis whereas its N322Q mutant favors the nonfucosylated GlcNAc as the acceptor (Fan et al., 2012). The ENGase-catalyzed transglycosylation has further been improved through the efficient synthesis of sugar oxazolines in H2O with 2-chloro-1,3-dimethylimidazolinium chloride (Noguchi et al., 2009) or 2-chloro-1,3-dimethyl-1H-benzimidazol-3-ium chloride (CDMBI) as a dehydrative condensing agent (Noguchi et al., 2012).

Chemoenzymatic glycosylation of IgG antibodies has been reported with Endo-A, yeast-produced IgG-Fc bearing high mannose-type glycans as the acceptor and Man3GlcNAc-oxazolines as the donor substrate (Wei et al., 2008). Complete transglycosylation of the IgG-Fc (N232A and N322Q) can also attach a Man3GlcNAc tetrasaccharide to a fucosylated GlcNAc-containing Fc (Fan et al., 2012) whereas none of Endo-D, Endo-A and their mutants can transfer intact complex-type N-glycan to either fucosylated or non-fucosylated GlcNAc-containing Fc. The Endo-M-N175Q mutant has recently been shown to act on proteins with a broad range of molecular weight including IgG (Fig. 5) despite a preference of low molecular weight proteins as acceptors. Endo-S mutants (Endo-S D233A and D233Q) are the first ENGase-based glycosynthases applicable for remodeling of IgG glycoforms with fucosylated and nonfucosylated full-length complex-type glycans using rituximab (Huang et al., 2012). The D184M and D184Q mutants of Endo-S2 from Streptococcus pyogenes NZ131 (serotype M49) have been reported to have more potent transglycosylation activity and more relaxed substrate specificity than the Endo-S-D233Q mutants (Li et al., 2016). Among the high mannose-, hybrid-, and complex-type N-glycan substrates, Endo-S2 prefers the complex-type over the other two types. The lower concentrations of sugar oxazolines and the shorter incubation times would be beneficial to reduce the risk of unwanted side reactions to the transglycosylation products.

Industrial scale production of homogeneous antibody glycoforms by chemoenzymatic glycoengineering would require large-scale production of homogeneous oligosaccharide substrates, simplification of synthesis for sugar oxazolines, and enhancement of the transglycosylation efficiency of glycosynthases. The production of complex-type oligosaccharides has recently been developed using egg yolk (Sun et al., 2014). The one-step synthesis of sugar oxazolines from unprotected sugars has been discovered by the use of CDMBI (Noguchi et al., 2012). The transglycosylation efficiency of the ENGase-based glycosynthases has been improved by systematic mutagenesis at the critical residues of various ENGases (Li et al., 2016). The development of this glycoengineering technology opens a new avenue to glycoform remodeling for therapeutic purposes.

CONCLUSION

IgG-Fc glycoengineering contributes to the development of next-generation therapeutic IgG antibodies with enhanced or silenced Fc effector functions. With the success of nonfucosylated IgG antibodies in the clinic, glycoengineered IgG antibodies have proven to be efficacious and devoid of immunogenicity in vivo as long as the Fc bears naturally occurring human-type glycans, in contrast to mutant forms of antibodies. Therefore, a range of glycan structures from monosaccharide GlcNAc to fully sialylated biantennary complex-type are to be explored for the design of homogeneous IgG glycoforms as therapeutic antibodies. Chemoenzymatic glycoengineering is a robust approach for remodeling of IgG-Fc glycoforms. It should be noted that gain- or loss-of-function may occur in a subclass-dependent manner as human IgG consists of four subclasses with differing abilities to activate different FcyRs and complement (Kao et al., 2015; Niwa et al., 2005). As the structural basis for the enhanced ADCC activity of nonfucosylated IgG antibodies has been elucidated, the carbohydrate–carbohydrate interactions between IgG-Fc and FcyR may also be a key issue for the design of novel glycoengineered IgG antibodies. On the other hand, E. coli-produced aglycosylated IgG antibodies with compromised effector functions can be exploited as neutralizing, agonist and antagonist antibodies for a wide range of diseases including cancers and autoimmune diseases. Bypassing glycosylation contributes to shorter bioprocess development and running times, without concerns about glycosylation heterogeneity as COAs, and is expected to substantially reduce production costs. The high costs of therapeutic antibodies have imposed financial pressures on national and private health care bodies. Blocking/neutralizing antibodies anti-PD-1 IgG nivolumab (Fig. 1B), anti-VEGF IgG bevacizumab (Fig. 1C), and anti-TNFα IgG infliximab are among largest selling pharmaceuticals that could maintain their efficacies in an aglycosylated format as demonstrated by the licensed therapeutic Fab fragments cetolizumab and ranibizumab specific for TNFα and VEGF-A, respectively. Thus, glycoengineering provides strategies to optimize the safety, functionality, and efficacy of therapeutic IgG antibodies as more affordable treatment options in the next decade.

ACKNOWLEDGEMENTS

Kaito Mimura (University of Tokyo) is thanked for his assistance with the molecular models of Figs. 2–4. This study was supported by JSPS KAKENHI grant No. 23590578 (Y. M.), the Science Foundation Ireland Starting Investigator Research grant (SFI SirG) under grant No. 13/SIRG/2164 (R. S.), and EU FP7 program HighGlycan grant No. 278535 (R. O.).
ABBREVIATIONS

ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; CDMB1, 2-chloro-1,3-dimethyl-1H-benzimidazol-3-ium chloride; CHO, Chinese hamster ovary; ENGase, endo-β-N-acetylglucosaminidase; Fab, fragment for antigen binding; Fc, fragment crystallizable; FcγR, receptor for Fc portion of IgG; FcRn, neonatal Fc receptor; FUT8, α(1-6)-fucoyltransferase; GlcNAc, N-acetylglucosamine; GnT-III, N-acetylglucosaminyltransferase III, HILIC, hydrophilic interaction liquid chromatography; IgG, immunoglobulin G; IVIG, intravenous immunoglobulin; NeuAc, N-acetylenuraminic acid; NeuGc, N-glycolylneuraminic acid; NMR, nuclear magnetic resonance; UPLC, ultra performance liquid chromatography; SNA, Sambucus nigra agglutinin

COMPLIANCE WITH ETHICS GUIDELINES

Yusuke Mimura, Toshihiko Katoh, Radka Saldova, Rosin O’Flaherty, Tomonori Izumi, Yuka Mimura-Kinuma, Toshiaki Utsunomiya, Yoichi Mizukami, Kenji Yamamoto, Tsuneo Matsumoto, and Pauline M. Rudd declare that they have no conflicts of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

OPEN ACCESS

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

REFERENCES

Ahmed AA, Giddens J, Pincetic A, Lomino JV, Ravetch JV, Wang LX, Bjorkman PJ (2014) Structural characterization of anti-inflammatory immunoglobulin G Fc proteins. J Mol Biol 426:3166–3179
Albert H, Collin M, Dudziak D, Ravetch JV, Nimmerjahn F (2008) In vivo enzymatic modulation of IgG glycosylation inhibits autoimmunity in an IgG subclass-dependent manner. Proc Natl Acad Sci USA 105:15005–15009
Allhorn M, Collin M (2009) Sugar-free antibodies—the bacterial solution to autoimmunity? Ann N Y Acad Sci 1173:664–669
Anthony RM, Kobayashi T, Wermeling F, Ravetch JV (2011) Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. Nature 475:110–113
Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV (2008a) Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 320:373–376
Anthony RM, Wermeling F, Karlsson MC, Ravetch JV (2008b) Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci USA 105:19571–19578
Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA (2006) The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 25:21–50
Ashwell G, Harford J (1982) Carbohydrate-specific receptors of the liver. Annu Rev Biochem 51:531–554
Barb AW, Brady EK, Prestegard JH (2009) Branch-specific sialylation of IgG-Fc glycans by ST6Gal-I. Biochemistry 48:9705–9707
Barb AW, Meng L, Gao Z, Johnson RW, Moremen KW, Prestegard JH (2012) NMR characterization of immunoglobulin G Fc glycan motion on enzymatic sialylation. Biochemistry 51:4618–4626
Baruah K, Bowden TA, Krishna BA, Dwek RA, Crispin M, Scanlan CN (2012) Selective deactivation of serum IgG: a general strategy for the enhancement of monoclonal antibody receptor interactions. J Mol Biol 420:1–7
Bazin R, Lemieux R, Tremblay T (2006) Reversal of immune thrombocytopenia in mice by cross-linking human immunoglobulin G with a high-affinity monoclonal antibody. Br J Haematol 135:97–100
Bones J, Mittermayr S, O’Donoghue N, Guttmann A, Rudd PM (2010) Ultra performance liquid chromatographic profiling of serum N-glycans for fast and efficient identification of cancer associated alterations in glycosylation. Anal Chem 82:10208–10215
Borrok MJ, Jung ST, Kang TH, Monzino AF, Georgiou G (2012) Revisiting the role of glycosylation in the structure of human IgG Fc. ACS Chem Biol 7:1596–1602
Bruhns P, Samuelsson A, Pollard JW, Ravetch JV (2003) Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity 18:573–581
Campbell IK, Miescher S, Branch DR, Mott PJ, Lazarus AH, Han D, Maraskovsky E, Zuercher AW, Neschadim A, Leontyev D et al (2014) Therapeutic effect of IVIG on inflammatory arthritis in mice is dependent on the Fc portion and independent of sialylation or basophils. J Immunol 192:5031–5038
Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, Murphy BA, Satinover SM, Hosen J, Mauro D et al (2008) Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med 358:1109–1117
Collin M, Shannon O, Bjorck L (2008) IgG glycan hydrolysis by a bacterial enzyme as a therapy against autoimmune conditions. Proc Natl Acad Sci USA 105:4265–4270
Crispin M, Yu X, Bowden TA (2013) Crystal structure of sialylated IgG Fc: implications for the mechanism of intravenous immunoglobulin therapy. Proc Natl Acad Sci USA 110:E3544–3546
Davies AM, Jefferis R, Sutton BJ (2014a) Crystal structure of deglycosylated human IgG4-Fc. Mol Immunol 62:46–53
Davies AM, Rispens T, Ooijevaar-de Heer P, Gould HJ, Jefferis R, Aalberse RC, Sutton BJ (2014b) Structural determinants of unique properties of human IgG4-Fc. J Mol Biol 426:630–644
Debre M, Bonnet MC, Fridman WH, Carosella E, Philippe N, Reinert P, Vilmer E, Kaplan C, Teillaud JL, Griscelli C (1993) Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. Lancet 342:945–949
Deisenhofer J (1981) Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-A resolution. Biochemistry 20:2361–2370
Delord JP, Tabernero J, Garcia-Carbonero R, Cervantes A, Gomez-Roca C, Berge Y, Capdevila J, Paz-Ares L, Roda D, Delmar P et al (2014) Open-label, multicentre expansion cohort to evaluate...
Glycoengineering for next-generation therapeutic antibodies

REVIEW

Pharmacokinetics of monoclonal antibodies and Fc fusion proteins. Eur J Pharm Biopharm 100:94–100

Holland M, Yagi H, Takahashi N, Kato K, Savage CO, Goodall DM, Jefferis R (2006) Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA-associated systemic vasculitis. Biochim Biophys Acta 1760:669–677

Huang W, Giddens J, Fan SQ, Toonstra C, Wang LX (2012) Chemoenzymatic glycoengineering of intact IgG antibodies for gain of functions. J Am Chem Soc 134:12308–12318

Iida S, Misaka H, Inoue M, Shibata M, Nakano R, Yamane-Ohruki N, Waktiani M, Yano K, Shitara K, Satoh M (2006) Nonfucosylated therapeutic IgG1 antibody can evade the inhibitory effect of serum immunoglobulin G on antibody-dependent cellular cytotoxicity through its high binding to FcgammarIIa. Clin Cancer Res 12:2879–2887

Ishida T, Joh T, Uike N, Yamamoto K, Utsunomiya A, Yoshida S, Saburi Y, Miyamoto T, Takemoto S, Suzuzhima H et al (2012) Defucosylated anti-CCR4 monoclonal antibody (KWO-716) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. J Clin Oncol 30:837–842

Iwamoto T, Okamoto A, Ishinaga H, Shimizu K, Gayle AA, Arai N, Takeuchi K, Okuda M (2016) A novel approach to predict cetuximab-induced hypersensitivity reaction: detection of drug-specific IgE on basophils. Cancer Med 5:1004–1012

Jefferis R (2009) Glycosylation as a strategy to improve antibody-based therapeutics. Nat Rev Drug Discov 8:226–234

Jefferis R (2012) Isotype and glycoform selection for antibody therapeutics. Arch Biochem Biophys 526:159–166

Jefferis R (2017) Characterization of biosimilar biologics: the link between structure and functions. In: Endrenyi L, Declerck P, Chow S-C (eds) Drugs and the pharmaceutical sciences. CRC Press, Boca Raton, pp 109–149

Ju MS, Jung ST (2014) Aglycosylated full-length IgG antibodies: steps toward next-generation immunotherapeutics. Curr Opin Biotechnol 30:128–139

Ju MS, Na JH, Yu YG, Kim JY, Jeong C, Jung ST (2015) Structural consequences of aglycosylated IgG Fc variants evolved for FcgammarII binding. Mol Immunol 67:350–356

Jung ST, Kang TH, Kelton W, Georgiou G (2011) Bypassing glycosylation: engineering aglycosylated full-length IgG antibodies for human therapy. Curr Opin Biotechnol 22:858–867

Jung ST, Reddy ST, Kang TH, Borrok MJ, Sandlje I, Tucker PW, Georgiou G (2010) Aglycosylated IgG variants expressed in bacteria that selectively bind FcgammarII potentiate tumor cell killing by monocye-dendritic cells. Proc Natl Acad Sci USA 107:604–609

Kanda Y, Yamada T, Mori K, Okazaki A, Inoue M, Kitajima-Miyama K, Kunimoto CR, Nakano R, Yano K, Kakita S et al (2007) Comparison of biological activity among nonfucosylated therapeutic IgG1 antibodies with three different N-linked Fc oligosaccharides: the high-mannose, hybrid, and complex types. Glycobiology 17:104–118

Kaneyo K, Nimmentjahn F, Ravetch JV (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313:670–673

Kao D, Danzer H, Collin M, Gross A, Eichler J, Stambuk J, Lauc G, Lux A, Nimmentjahn F (2015) A monosaccharide residue is sufficient to maintain mouse and human IgG subclass activity and
directs IgG effector functions to cellular Fc receptors. Cell Rep 13:2376–2385
Kato T, Fujita K, Takeuchi M, Kobayashi K, Natsuka S, Ikura K, Kumagai H, Yamamoto K (2002) Identification of an endo-beta-N-acetylgalactosaminidase gene in Caenorhabditis elegans and its expression in Escherichia coli. Glycobiology 12:581–587
Kobayashi S, Kiyosada T, Shoda S-I (1996) Enzymatic synthesis of chondroitin and its derivatives catalyzed by hyaluronidase. J Am Chem Soc 118:13113–13114
Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M (1997) Fc gammaRllla-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRllla, independently of the Fc gammaRllla-48L/R/H phenotype. Blood 90:1109–1114
Krapp S, Mimura Y, Jefferis R, Huber R, Sondermann P (2003) Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. J Mol Biol 325:979–989
Kurogochi M, Mori M, Osumi K, Tojino M, Sugawara S, Takashima S, Kato T, Fujita K, Takeuchi M, Kobayashi K, Natsuka S, Ikura K, Takashima S, Li H, Sethuraman N, Stadheim TA, Zha D, Prinz B, Ballew N, Bobrowicz P, Choi BK, Cook WJ, Cukan M et al (2006) Expression of the Fc gammaRIIIa-48L/R/H phenotype. Nat Biotechnol 24:210
Lee SJ, Evers S, Roeder D, Parlow AF, Risteli J, Risteli L, Lee YC, Feizi T, Langen H, Nussenzweig MC (2002) Mannose receptor-mediated regulation of serum glycoprotein homeostasis. Science 295:1898–1901
Leontyev D, Katsman Y, Branch DR (2012a) Mouse background and therapeutic monoclonal antibodies: from the Bench to the Clinic. Wiley, Hoboken, pp 67–89
Lee SJ, Evers S, Roeder D, Parlow AF, Risteli J, Risteli L, Lee YC, Feizi T, Langen H, Nussenzweig MC (2002) Mannose receptor-mediated regulation of serum glycoprotein homeostasis. Science 295:1898–1901
Leontyev D, Katsman Y, Ma XZ, Miescher S, Kasermann F, Branch DR (2012b) Sialylation-independent mechanism involved in the amelioration of murine immune thrombocytopenia using intravenous gammaglobulin. Transfusion 52:1799–1805
Li B, Zeng Y, Hauser S, Song H, Wang LX (2005) Highly efficient endoglycosidase-catalyzed synthesis of glycopeptides using oligosaccharide oxazolines as donor substrates. J Am Chem Soc 127:9692–9693
Li H, Sethuraman N, Stadheim TA, Zha D, Prinz B, Ballew N, Bobrowicz P, Choi BK, Cook WJ, Cukan M et al (2006) Optimization of humanized IgGs in glycoengineered Pichia pastoris. Nat Biotechnol 24:210–215
Li T, Tong X, Yang Q, Giddens JP, Wang LX (2016) Glycosynthase mutants of endoglycosidase S2 show potent transglycosylation activity and remarkably relaxed substrate specificity for antibody glycosylation remodeling. J Biol Chem 291:16508–16518
Lin CW, Tsai MH, Li ST, Tsai TI, Chu KC, Liu YC, Lai MY, Wu CY, Tseng YC, Shivatare SS et al (2015) A common glycan structure on immunoglobulin G for enhancement of effector functions. Proc Natl Acad Sci USA 112:10611–10616
Liu L (2015) Antibody glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins. J Pharm Sci 104:1866–1884
Liu L (2017) Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins. Protein Cell. doi:10.1007/s13238-017-0408-4
Liu L, Stadheim A, Hamulo L, Pittman T, Wang W, Zha D, Hochman J, Pruksavarantont T (2011) Pharmacokinetics of IgG1 monoclonal antibodies produced in humanized Pichia pastoris with specific glycoforms: a comparative study with CHO produced materials. Biologicals 39:205–210
Lund J, Tanaka T, Takahashi N, Sarmay G, Arata Y, Jefferis R (1990) A protein structural change in aglycosylated IgG3 correlates with loss of huFc gamma R1 and huFc gamma R111 binding and/or activation. Mol Immunol 27:1145–1153
Maenaka K, van der Merwe PA, Stuart DI, Jones EY, Sondermann P (2001) The human low affinity Fc gamma receptors IIa, IIb, and III bind IgG with fast kinetics and distinct thermodynamic properties. J Biol Chem 276:44988–44904
Mariotte D, Dupont B, Gervais R, Galais MP, Laroche D, Tranchant A, Comby E, Bouhier-Leporrier K, Reimund JM, Le Mauff B (2011) Anti-cetuximab IgE ELISA for identification of patients at a high risk of cetuximab-induced anaphylaxis. Mabs 3:396–401
Matsumiya S, Yamaguchi Y, Saito J, Nagano M, Sasakawa H, Otaki S, Sato M, Shitara K, Kato K (2007) Structural comparison of fucosylated and nonfucosylated Fc fragments of human immunoglobulin G1. J Mol Biol 368:767–779
Mimura Y, Ashton PR, Takahashi N, Harvey DJ, Jefferis R (2007) Glycosylation profiles between Fab and Fc of a human IgG protein studied by electrospray ionization mass spectrometry. J Immunol Methods 326:116–126
Mimura Y, Church S, Ghirlando R, Ashton PR, Dong S, Goodall M, Lund J, Jefferis R (2000) The influence of glycosylation on the thermal stability and effector function expression of human IgG1-Fc: properties of a series of truncated glycoforms. Mol Immunol 37:697–706
Mimura Y, Jefferis R, Mimura-Kimura Y, Abrahams J, Rudd PM (2009) Glycosylation of therapeutic IgGs. In: An Z (ed) Therapeutic monoclonal antibodies: from the Bench to the Clinic. Wiley, Hoboken, pp 67–89
Mimura Y, Kelly RM, Unwin L, Albrecht S, Jefferis R, Goodall M, Mizukami Y, Mimura-Kimura Y, Matsumoto T, Ueoka H et al (2016) Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines. J Immunol Methods 428:30–36
Mimura Y, Sondermann P, Ghirlando R, Lund J, Young SP, Goodall M, Jefferis R (2001) Role of oligosaccharide residues of IgG1-Fc in Fc gamma Rllb binding. J Biol Chem 276:45539–45547
Mizushima T, Yagi H, Takemoto E, Shibata-Koyama M, Isoda Y, Iida S, Masuda K, Sato M, Kato K (2011) Structural basis for improved efficacy of therapeutic antibodies on defucosylation of their Fc glycans. Genes Cells 16:1071–1080
Mossner E, Brunker P, Moser S, Puntener U, Schmidt C, Herter S, Grau R, Gerdes C, Nopora A, van Puijenbroek E et al (2010) Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood 115:4393–4402
Muramatsu H, Tachikui H, Ushida H, Song X, Qiu Y, Yamamoto S, Muramatsu T (2001) Molecular cloning and expression of endo-beta-N-acetylgalactosaminidase D, which acts on the core structure of complex type asparagine-linked oligosaccharides. J Biochem 129:923–928

© The Author(s) 2017. This article is an open access publication
Glycoengineering for next-generation therapeutic antibodies

Niwa R, Natsume A, Uehara A, Wakitani M, Iida S, Uchida K, Satoh M, Shitara K (2005) IgG subclass-independent improvement of antibody-dependent cellular cytotoxicity by fusoc removal from Asn297-linked oligosaccharides. J Immunol Methods 306:151–160

Noguchi M, Fujieda T, Huang WC, Ishihara M, Kobayashi A, Shoda S-I (2012) A practical one-step synthesis of 1,2-oxazoline derivatives from unprotected sugars and its application to chemoenzymatic beta-N-acetylglucosaminidation of disialo-oligosaccharide. Helv. Chim. Acta 95:1928–1936

Noguchi M, Tanaka T, Gyakushi H, Kobayashi A, Shoda S (2009) Efficient synthesis of sugar oxazolines from unprotected N-acetyl-2-aminosaccharides using chloroformimidinium reagent in water. J Org Chem 74:2210–2212

Nose M, Wigzell H (1983) Biological significance of carbohydrate chains on monoclonal antibodies. Proc Natl Acad Sci USA 80:6636–6636

Okazaki A, Shoji-Hosaka E, Nakamura K, Wakitani M, Uchida K, Kakita S, Tsumoto K, Kumagai I, Shitara K (2004) Fusoc depletion from human IgG1 oligosaccharide enhances binding enthalpy and association rate between IgG1 and FcgammaRIIa. J Mol Biol 336:1239–1249

Othy S, Topcu S, Saha C, Kolhappali P, Lacroix-Demsazes S, Kasermann F, Miescher B, Bayry J, Kaveri SV (2014) Sialylation may be dispensable for reciprocal modulation of helper T cells by intravenous immunoglobulin. Eur J Immunol 44:2059–2063

Padian EA (1990) X-ray diffraction studies of antibody constant regions. In: Metzger H (ed) Fc receptors and the action of antibodies. American Society for Microbiology, Washington, DC, pp 12–30

Pound JD, Lund J, Jefferis R (1993) Aglycosylated chimaeric human IgG3 can trigger the human phagocyte respiratory burst. Mol Immunol 29:233–241

Pucic M, Knezievic A, Vidic J, Adamczyk B, Novokmet M, Polasek O, Gomik O, Supraha-Goreta S, Wormald MR, Redzic I et al (2011) High throughput purification and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations. Mol Cell Proteomics 10(M111):010090

Qian J, Liu T, Yang L, Daus A, Crowley R, Zhou Q (2007) Structural characterization of N-linked oligosaccharides on monoclonal antibody cetuximab by the combination of orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole–quadrupole time-of-flight tandem mass spectrometry and sequential enzymatic digestion. Anal Biochem 364:8–18

Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, Munz C, Nimmerjahn F, Dalalas MC, Lunemann JD (2015) Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity. J Clin Invest 125:4160–4170

Radaev S, Motyka S, Fridman WH, Sautes-Fridman C, Sun PD (2001) The structure of a human type III Fcgamma receptor in complex with Fc. J Biol Chem 276:16469–16477

Radcliffe CM, Arnold JN, Suter DM, Wormald MR, Harvey DJ, Royle L, Mimura Y, Kinoya Y, Sim RB, Inoges S et al (2007) Human follicular lymphoma cells contain oligomannose glycans in the antigen-binding site of the B-cell receptor. J Biol Chem 282:7405–7415

Raju TS, Briggs JB, Borge SM, Jones AJ (2000) Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics. Glycobiology 10:477–486

Raymond C, Robotham A, Spearman M, Butler M, Kelly J, Durocher Y (2015) Production of alpha2,6-sialylated IgG1 in CHO cells. MAbs 7:571–583

Roopenian DC, Akiles S (2007) FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 7:715–725

Royle L, Radcliffe CM, Dwek RA, Rudd PM (2006) Detailed structural analysis of N-glycans released from glycoproteins in SDS-PAGE gel bands using HPLC combined with exoglycosidase array digestion methods. Methods Mol Biol 347:125–143

Rudd PM, Dwek RA (1997) Glycosylation: heterogeneity and the 3D structure of proteins. Crit Rev Biochem Mol Biol 32:1–100

Sarmay G, Lund J, Rozsnyay Z, Gergely J, Jefferis R (1992) Mapping and comparison of the interaction sites on the Fc region of IgG responsible for triggering antibody dependent cellular cytotoxicity (ADCC) through different types of human Fc gamma receptor. Mol Immunol 29:633–639

Sazinsky SL, Ott RG, Silver NW, Tidor B, Ravetch JV, Wittrup KD (2008) Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. Proc Natl Acad Sci USA 105:20167–20172

Scalllon BJ, Tam SH, McCarthy SG, Cai AN, Raju TS (2007) Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality. Mol Immunol 44:1524–1534

Sehn LH, Assouline SE, Stewart DA, Mangel J, Gascoyne RD, Fine G, Frances-Lasserre S, Carlile DJ, Crump M (2012) A phase 1 study of obinutuzumab induction followed by 2 years of maintenance in patients with relapsed CD20-positive B-cell malignancies. Blood 119:5118–5125

Sehn LH, Goy A, Offner FC, Martellini G, Caballero MD, Gadeberg O, Baetz T, Zelenetz AD, Gaidano G, Fayad LE et al (2015) Randomized phase II trial comparing obinutuzumab (GA101) with rituximab in patients with relapsed CD20 + indolent B-Cell non-Hodgkin lymphoma: final analysis of the GAUSS study. J Clin Oncol 33:3473–3474

Shibata-Koyama M, Iida S, Okazaki A, Mori K, Kitajima-Miyama K, Saitou S, Kakita S, Kanda Y, Shitara K, Kato K et al (2009) The N-linked oligosaccharide at Fc gamma RIIa Asn45: an inhibitory element for high Fc gamma RIIIa binding affinity to IgG glycoforms lacking core fusoc. Glycobiology 19:126–134

Shields RL, Lai J, Keck R, O’Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fusoc on human IgG1N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277:26733–26740

Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li B et al (2001) High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem 276:6591–6604

Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Sato M, Yamasaki M et al (2003) The absence of fusoc but not the presence of galactose or bisecting N-acetylgalactosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–3473

© The Author(s) 2017. This article is an open access publication.
Simmons LC, Reilly D, Klimowski L, Raju TS, Meng G, Sims P, Hong K, Shields RL, Damico LA, Rancatore P et al (2002) Expression of full-length immunoglobulins in Escherichia coli: rapid and efficient production of aglycosylated antibodies. J Immunol Methods 263:133–147
Sonnermann P, Huber R, Jacob U (1999) Crystal structure of the soluble form of the human fçgamma-receptor lib: a new member of the immunoglobulin superfamily at 1.7 Å resolution. EMBO J 18:1095–1103
Sonnermann P, Huber R, Oosthuizen V, Jacob U (2000) The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc gammaRIII complex. Nature 406:267–273
Sonnermann P, Jacob U (1999) Human Fçgamma receptor lib expressed in Escherichia coli reveals IgG binding capability. Biol Chem 370:717–721
Sun B, Bao W, Tian X, Li M, Liu H, Dong J, Huang W (2014) A simplified procedure for gram-scale production of sialylglycopeptide (SGP) from egg yolk and subsequent semi-synthesis of Man3GlcNAc oxazoline. Carbohydr Res 396:62–69
Takegawa K, Tabuchi M, Yamaguchi S, Kondo A, Kato I, Ihahaha S (1995) Synthesis of neoglycoproteins using oligosaccharide-transfer activity with endo-beta-N-acetylglucosaminidase. J Biol Chem 270:3094–3099
Takegawa K, Yamabe K, Fujita K, Tabuchi M, Mita M, Izu H, Watanabe A, Asada Y, Sano M, Kondo A et al (1997) Cloning, sequencing, and expression of Arthrobacter protophormiae endo-beta-N-acetylglucosaminidase in Escherichia coli. Arch Biochem Biophys 338:22–28
Takeuchi M, Takasaki S, Miyazaki H, Kato T, Hoshi S, Kochibe N, Kobata A (1988) Comparative study of the asparagine-linked sugar chains of human erythrocytopenins purified from urine and the culture medium of recombinant Chinese hamster ovary cells. J Biol Chem 263:3657–3663
Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varik N, Varik A, Muchmore E (2003) Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc Natl Acad Sci USA 100:12045–12050
Tao MH, Morrison SL (1989) 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 143:2595–2601
Trinath J, Hegde P, Sharma M, Maddur MS, Rabin M, Vallat JM, Magy L, Balaji KN, Kaveri SV, Bayry J (2013) Intravenous immunoglobulin expands regulatory T cells via induction of cyclooxygenase-2-dependent prostaglandin E2 in human dendritic cells. Blood 122:1419–1427
Umana P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE (1999) Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nat Biotechnol 17:176–180
Umekawa M, Li C, Higashiyama T, Huang W, Ashida H, Yamamoto K, Wang LX (2010) Efficient glycosynase mutant derived from Mucor hiemalis endo-beta-N-acetylglucosaminidase capable of transferring oligosaccharide from both sugar oxazoline and natural N-glycan. J Biol Chem 285:511–521
van den Eijnden DH, Joziassse DH, Dorland L, van Halbeek H, Vliegenthart JF, Schmid K (1980) Specificity in the enzymic transfer of sialic acid to the oligosaccharide branches of b1- and triantennary glycopeptides of alpha 1-acid glycoprotein. Biochem Biophys Res Commun 92:839–845
Wang B, Yan L, Yao Z, Roskos LK (2017) Population pharmacokinetics and pharmacodynamics of benralizumab in healthy volunteers and patients with asthma. CPT Pharmacomet Syst Pharmacol. (6):249–257
Wei Y, Li C, Huang W, Li B, Strome S, Wang LX (2008) Glycoengineering of human IgG1-Fc through combined yeast expression and in vitro chemoenzymatic glycosylation. Biochemistry 47:10294–10304
Woof JM, Burton DR (2004) Human antibody-Fc receptor interactions illuminated by crystal structures. Nat Rev Immunol 4:89–99
Wright A, Tao MH, Kabat EA, Morrison SL (1991) Antibody variable region glycosylation: position effects on antigen binding and carbohydrate structure. EMBO J 10:2717–2723
Wu J, Edberg JC, Redecha PB, Bansal V, Guyre PM, Coleman K, Salmon JE, Kimberly RP (1997) A novel polymorphism of FçgammaRIIa (CD16) alters receptor function and predisposes to autoimmune disease. J Clin Invest 100:1059–1070
Wu SJ, Luo J, O’Neil KT, Kang J, Lacy ER, Canziani G, Baker A, Huang M, Tang QM, Raju TS et al (2010) Structure-based engineering of a monoclonal antibody for improved solubility. Protein Eng Des Sel 23:643–651
Yamaguchi Y, Nishimura M, Nagano M, Yagi H, Sasakawa H, Uchida K, Shiitara K, Kato K (2006) Glycoform-dependent conformational alteration of the Fc region of human immunoglobulin G1 as revealed by NMR spectroscopy. Biochim Biophys Acta 1760:693–700
Yamamoto K, Kadowaki S, Fujisaki M, Kumagai H, Tochikura T (1994) Novel specificities of Mucor hiemalis endo-beta-N-acetylglucosaminidase acting complex asparagine-linked oligosaccharides. Biosci Biotechnol Biochem 58:72–77
Yamamoto K, Utsunomiya A, Tobinai K, Tsukasaki K, Uike N, Uozumi K, Yamaguchi K, Yamada Y, Hanada S, Tomura K et al (2010) Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. J Clin Oncol 28:1591–1598
Yamane-Ohruki N, Kinoshita S, Inoue-Urakubo M, Kusunoki M, Iida S, Nakano R, Wakitani M, Niwa R, Sakurada M, Uchida K et al (2004) Establishment of FUT8 knockout Chinese hamster ovary cells: an ideal host cell line for producing completely defucosylated antibodies with enhanced antibody-dependent cellular cytotoxicity. Biotechnol Bioeng 87:614–622
Yu X, Vasiljevic S, Mitchell DA, Crispin M, Scanlan CN (2013) Dissecting the molecular mechanism of IVig therapy: the interaction between serum IgG and DC-SIGN is independent of antibody glycoform or Fc domain. J Mol Biol 425:1253–1258
Zhang P, Woen S, Wang T, Liu B, Zhao S, Chen C, Yang Y, Song Z, Wormald MR, Yu C et al (2016) Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs. Drug Discov Today 21:740–765
Zhu A, Hurst R (2002) Anti-N-glycolyneuraminic acid antibodies identified in healthy human serum. Xenotransplantation 9:376–381