Methods for the glycosylation analysis of therapeutic antibodies

Reusch, D.

2015

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Reusch, D. (2015). *Methods for the glycosylation analysis of therapeutic antibodies*.

Chapter 2:

Fc glycans of therapeutic antibodies as critical quality attributes (CQAs)

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Abstract

Critical quality attributes (CQA) are physical, chemical, biological or microbiological properties or characteristics that must be within an appropriate limit, range, or distribution to ensure the desired product quality, safety, and efficacy. For monoclonal antibody therapeutics that rely on Fc-mediated effector function for their clinical activity, the terminal sugars of Fc glycans have been shown to be critical for safety or efficacy. Different glycosylation variants have also been shown to influence the pharmacodynamic and pharmacokinetic behavior while other Fc glycan structural elements may be involved in adverse immune reactions. This review focuses on the role of Fc glycans as CQAs. Fc glycan information from published literature is summarized and evaluated for impact on patient safety, immunogenicity, bioactivity and pharmacodynamics/pharmacokinetics.

Introduction

Therapeutic monoclonal antibodies recognizing cell surface expressed antigens, can engage Fcγ receptors (FcγR) on effector cells (monocytes, macrophages, natural killer cells, neutrophils, eosinophils and dendritic cells), or bind to C1q, and elicit immune effector functions such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) [1]. Additionally, direct apoptosis may be enhanced by effector cell dependent cross-linking [2]. The family of Fcγ receptors mediating these activities includes FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and FcγRIIIb [60-62]. These receptors differ in their antibody binding affinities (FcγRI binds with higher affinity to IgG than FcγRII and FcγRIII) and can be characterized as either activating (FcγRI, FcγRIIa, FcγRIII), or inhibitory (FcγRIIb).

Antibodies (also commonly referred to as immunoglobulins, or IgG) consist of two heavy chains and two light chains. Each heavy chain contains three domains, CH1, CH2 and CH3 with a hinge region between CH1 and CH2. The heavy chain contains biantennary N-glycan structures linked to Asn297 in the CH2 domain [3]. The two glycan chains on each of the CH2 domains are often different and contribute to the asymmetrical binding of the Fc to the Fc-receptors [4-7]. Fc glycosylation also stabilizes the Fc structure [8]. N-glycosylation is one of the most important post-translational modifications and often results in a remarkable heterogeneity of protein glycoforms [9;10]. Depending on the recombinant expression system, this may be a complex type, hybrid type or high-mannose type structure [11;12]. The use of mammalian expression systems generally results in complex type biantennary oligosaccharides in the Fc portions. These glycans may have a core fucose and a bisecting N-acetylglucosamine. Moreover, they can vary in terminal galactose and sialic acid content (see Figure 1 and Table 1) [10;13-15]. In addition, some IgGs contain additional N-glycans in the variable regions of the Fab portion. Fab N-glycans have been described to differ from the oligosaccharides of the Fc region in that they are generally more highly galactosylated and are more extensively decorated with sialic acids. About 15-20% of IgG are glycosylated in the Fab part with no impact to structure [10;16-18]. However, Fab glycosylation is not within the scope of this review.

Where the mechanism of action (MoA) involves immune-mediated effector function, these activities can be greatly influenced by the Fc glycosylation pattern. For example, a high degree of galactosylation can promote activation of the complement system *in vitro* by increasing C1q binding and CDC, and could have modest effects on ADCC [19-21]. It is also well-known that decreases in core-fucose levels lead to a pronounced increase in ADCC via increased affinity of IgG1 for FcγRIIIa on immune cells [22;23]. Recent findings indicate the lack of core fucose also promotes ADCP mediated by FcγRIIIa-positive monocytes and macrophages [24;25]. Differences in glycosylation patterns can also lead to differences in the pharmacodynamic and pharmacokinetic behavior [26;27]. In addition, IgG glycan structural elements such as α1-3 bound galactose and N-glycolylneuraminic acid may be involved in adverse immune reactions [28;29].
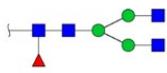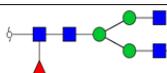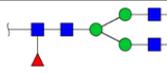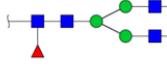
In some cases, a correlation has been established between FcγRIIIa phenotype and the clinical response, suggesting that ADCC activity could contribute to clinical efficacy [30-32]. These results prompted the idea

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to use glycoengineering as a means of improving efficacy [33]. Glycoengineering is a process used to manufacture antibodies lacking core fucose, which results in significantly higher binding affinity of human IgG1 antibodies to human FcγRIIIa, and consequently greatly enhanced ADCC activity. Shinkawa *et al.* showed a 50-fold increase in ADCC by IgG1s containing a high proportion of afucosylated Fc glycans [34]. Highly afucosylated antibodies can be generated by modifying the oligosaccharide biosynthesis activities in various ways. For example, overexpression of *N-acetylglucosamine-transferase III* (GnTIII) in the Golgi apparatus of the production cell line generates bisected oligosaccharide structures associated to the Fc constant region of the antibody and suppresses fucosylation. In such expression systems the level of GnTIII expression correlates with the generation of afucosylated IgG1 glycoforms and resulting enhanced ADCC activity [35]. Highly afucosylated antibodies can also be produced using a fucosyltransferase-deficient producer CHO cell line. In this technology, both FUT8 alleles are disrupted by homologous recombination, resulting in completely afucosylated recombinant IgGs [36;37].

A critical quality attribute (CQA) is defined as a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality, safety, and efficacy [38]Identification of CQAs is a component of the quality by design (QbD) approach to product development, along with identificatio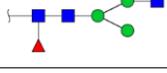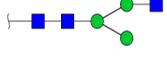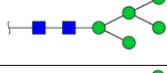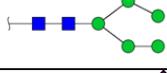n of the process parameters that affect these CQAs, the design and control of manufacturing via in-process and QC testing of critical quality and performance attributes of raw and in-process materials and processes that ensure manufacturing consistency. When the mode of action of an antibody involves effector function, glycosylation represents one of the main sources of heterogeneity with potential impact to safety and efficacy, making it a critical manufacturing parameter to monitor. Therefore, a thorough characterization of carbohydrate content, the structure of the carbohydrate chain, the oligosaccharides and glycosylation sites present on the antibody is critical [39].

This review focuses on product-specific clinical and nonclinical information, analytical and biological characterization, general and platform antibody knowledge, and published literature.

Figure 1. A schematic model of an IgG.

Table 1. Glycostructures commonly found in the Fc portion of a therapeutic antibody.

Name and composition	Classification	Structure	Exemplary relative abundance for a therapeutic antibody*
G0F [H3N4F1]	Complex, fucosylated		35.5
G1F [H4N4F1]	Complex, fucosylated		43.4
G2F [H5N4F1]	Complex, fucosylated		9.5
G1FS [H4N4F1S1]	Complex, fucosylated		0.2
G2S1F [H5N4F1S1]	Complex, fucosylated		0.7
G2S2F [H5N4F1S2]	Complex, fucosylated		0.1
G0 [H3N4]	Complex, non-fucosylated		4.6
G1 [H4N4]	Complex, non-fucosylated		3.3
G2 [H5N4]	Complex, non-fucosylated		0.3
G0F-N [H3N3F1]	Hybrid (monoantennary)		0.5
G0-N [H3N3]	Hybrid (monoantennary)		0.4
M5 [H5N2]	High mannose		1.5
M6 [H6N2]	High mannose		0.1
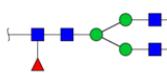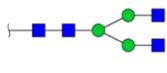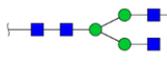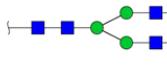
■ = N-acetylglucosamine ● = Mannose ○ = Galactose ▲ = Fucose ◆ = N-acetylneuraminic acid *see chapter 5

Critical quality attributes (CQAs)

The Annex to ICH Q8, defines critical quality attributes (CQA) as physical, chemical, biological or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality, safety/immunogenicity, efficacy and PK/PD.

Safety/Immunogenicity

The criticality assessment of a CQA with respect to safety and immunogenicity cannot be performed independent of qualitative and quantitative analysis of the various glycoforms and should take into account non-clinical e.g. IND-enabling tox studies, and clinical experience.

Safety is assessed based on non-clinical observations most often from studies on primates such as cynomolgus monkey, as well as observed clinical adverse events (AEs). These AEs can be target-related or non-target-related i.e. "off-target effects", anti-therapeutic antibody (ATA) related or ATA-independent.

AEs include infusion related reactions (IRR), injection site reactions, hypersensitivity, anaphylaxis, rash, neutropenia, thrombocytopenia and so on. IRR may be linked to IgG Fc glycosylation, as certain glycoforms can mediate complement activation. Furthermore, Fc glycans that affect Fc effector functions may impact on safety either by increasing otherwise low cell killing potential or through secondary effects such as cytokine release triggered by the activation of effector cells [41].

Biologic Activity (Efficacy)

While the glycosylation pattern is not known to affect the interaction of an antibody with its target, it can greatly influence effector functions by modulating binding to Fcγ receptors on immune cells [42-49]. Therefore, it is important to consider the contribution of Fc effector function to the mechanism of action (MoA) as part of the CQA assessment. This holds true independent of the classification of therapeutic antibodies based on their putative mechanisms of action (Class I MoA: cell-bound antigen with depletion; Class II MoA: cell-bound antigen with functional blocking; Class III MoA: soluble antigen with blocking) [22].

Clearance (PK/PD)

The neonatal Fc receptor (FcRn) plays a role in adult salvage of IgG. FcRn in the acidic endosomes bind to IgG internalized through transcytosis. The IgG is recycled to the cell surface and is released at the pH of blood (so it is prevented from lysosomal degradation). While aglycosylation has profound impacts to effector function, the interaction of IgG-Fc with FcRn is believed to be independent of Fc glycosylation [50]. Changes of the levels of the different glycoforms of the antibody as a component of circulation time are interpreted as arising from differences in clearance rates. In principle this could be due to differences in binding to the neonatal Fc receptor (FcRn) [51-55] or to differences in the clearing rate mediated by C-type lectins such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) or non-classical Fc-binding receptors including mannose-binding lectin 2 (MBL2), Dectin 1 and the macrophage mannose receptor (MMR) [56-60].

In general, the influence of Fc glycans on PK/PD is not well understood and the literature concerning the effects of Fc glycosylation variability on PK properties is ambiguous [61]. While some reports show no impact to PK for different glycospecies [62-66], others report changes in PK [67-69].

In contrast, Leabman *et al.* performed a comprehensive analysis regarding the impact of glycosylation on PK in cynomolgous monkey. This assessment includes data from aglycosylated antibodies generated via engineering (N297A, N297G) or by production in E. coli, an antibody with a (L234A/L235A) LALA mutation as well as glycoengineered antibodies. The antibodies targeted different antigen types, including highly expressed multi-transmembrane receptors, soluble cytokines, cell surface proteins and ligands. The results of this comprehensive study demonstrated that antibodies with differences in glycosylation that significantly alter FcγRIIIa binding, show no differences in PK [70]. The inclusion of a broad range of antibody targets in this study increases the likelihood that these findings are broadly applicable. Several studies have shown that glycoproteins i.e. IgG-Fc fusion proteins with terminal N-acetylglucosamine residues are thought to be cleared by means of the mannose receptor. However this clearance was mediated by N-acetylglucosamine in the receptor region and the Fc glycan ratio did not change [71;72].

Fc-glycans may not be accessible to asialoglycoprotein or mannose receptors that could mediate antibody clearance. Tao *et al.* showed that the serum half-life of aglycosylated IgG-Gln (IgG1 with one GlcNAc) in mice remains the same as for wild-type IgG1 [73]. However, Dong *et al.* found an increase in binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells [74].

Impact of Fc glycans

α1,3-Galactose (α1,3-gal)

IgG glycan structural elements such as α1,3 bound galactose may be involved in adverse immune reactions. In humans, anti-α1,3-gal IgG constitutes as much as 1% of circulating IgG. This glycovariant is unique amongst endogenous human antibodies because of its atypically high concentration in serum (30-100 µg/ml) and its presence in all humans [75]. Therapeutic monoclonal antibodies produced in murine myeloid cell lines like SP2/0 or NSO, contain α1,3-galactose structures on their Fc glycans [76-78]. It is generally accepted that CHO cells lack the biosynthetic machinery to synthesize glycoproteins with α1,3-gal moieties [79]. Bosques *et al.* determined that CHO 1-3,• -galactosyltransferase-1 is active and detected α1,3-galactose on two of their products; however, these products were CTLA4-Fc fusion proteins, and the α1,3-galactose was on the CTLA4 portion. The authors indicated that this may be a phenomenon resulting during single cell cloning of the production cell line, and that this does not occur in all CHO cells [80].

There are several reports discussing the role of α1,3-galactose epitope in xenotransplantation and on glycoproteins [81-83]. The most relevant case is that of cetuximab, a chimeric IgG1 monoclonal antibody against the epidermal growth factor receptor that is approved for use in colorectal cancer and squamous-cell carcinoma of the head and neck. While cetuximab is glycosylated on both its Fc and Fab domains, the α1,3-galactose structures are found only on the Fab portion [84]. This may be related to the sequence in the CDR and the fact that cetuximab is produced using SP2/0 cells.

Chung *et al.* reported a high prevalence of hypersensitivity reactions to cetuximab in some regions of the United States. In most of the subjects who exhibited a hypersensitivity reaction, IgE antibodies specific for the galactose-α-1,3-galactose on cetuximab were present in serum prior to therapy [85].

In contrast, van Lammerts *et al.* reported that anti α1,3-galactose IgE from allergic patients do not bind α1,3-galactosylated glycans on intact therapeutic antibody Fc domains. The authors showed that cetuximab was bound by α-Gal specific IgE antibodies in the serum of patients and that this binding is restricted to the Fab domain. Cetuximab Fc domains were not bound even though they contained detectable amounts of α1,3-galactose. This is believed to be due to the inaccessibility of the α-Gal moiety in the Fc domain, since binding is observed due to glycan exposure following proteolytic digest. The authors observed an increased affinity of α1,3-galactose IgE antibodies for glycostructures on the Fab domain bearing two α1,3-galactose moieties suggesting that therapeutic mAbs produced in rodent cell lines, and only glycosylated in their Fc domains, are not recognized by α1,3-galactose–specific IgE antibodies [86]. In conclusion, based on the literature, α1,3-galactose in Fc-glycans maybe a CQA concerning safety/immunogenicity. It should be closely monitored if Sp2/0 or NS0 cells are used for production of therapeutic antibodies.

β1,2-xylose and α1,3-fucose

Plants are attractive hosts for the manufacture of recombinant protein therapeutics as they are relatively inexpensive systems that can be readily scaled up. However, plant-derived monoclonal antibodies contain complex N-glycans containing β(1,2)-xylose and α1,3-fucose residues not present in human, and are therefore regarded as 'carbohydrate cross-reactive determinants' in IgE from sera of allergic patients [87]. The glycan-specific IgE show 23% prevalence and have been found at levels up to 71% in individuals with multiple pollen sensitivity. However, the clinical relevance of these reactive IgE remains unclear. Strasser *et al.* used RNAi to successfully silence XylT, the gene coding for • 1,2-xylotransferase, in tomato plants and demonstrated a patient-specific reduction in IgE reactivity in their studies [88]. Arabidopsis thaliana knockouts of the XylT (encoding • 1,2-xylotransferase) and FucT (encoding • 1,3 fucosyl transferase) genes have also been generated that are viable with no obvious phenotype. This represents a path forward in the production of protein biotherapeutics with human type N-glycosylation [89]. In conclusion if plants are used to produce therapeutic antibodies and the transferases are not silenced, β1,2-xylose and α1,3-fucose should be taken into account as CQAs.

N-glycolylneuramic acid (NGNA)

Glycans of therapeutic proteins that are produced in SP2/0, NSO, and to a much lesser extent in CHO cell lines are often modified with the non-human sialic acid N-glycolylneuraminic acid (Neu5Gc; NGNA) [90;91]. Humans do not synthesize NGNA as the gene encoding CMP-N-acetylneuraminic acid hydroxylase, which produces CMP-NGNA from CMP-NANA, is mutated [92]. It was previously thought that healthy humans did not react to NGNA; however, more recent findings suggest that all humans have anti-NGNA antibodies, some at levels up to 0.1-0.2% of circulating IgG. This may be due to dietary exposure, and the subsequent incorporation of NGNA into human proteins [93;94]. Padler-Karavani *et al.*

suggested that the ongoing antigen-antibody reaction may generate chronic inflammation, possibly contributing to the high frequency of diet-related carcinoma and other diseases in humans [95].

It has been demonstrated that many humans produce antibodies against oligosaccharide structures with terminal NGNA [96]. The potential impact of NGNA was evaluated by Durocher *et al.* who indicated that this immunogenicity can reduce efficacy due to rapid clearance of the biotherapeutic, or alternatively, prevent drug re-administration due to an undesirable immune response [97]. As these ATA represent the first obstacle to xenotransplantation in humans they are clinically significant from a safety perspective [98] [99]. Biotherapeutics produced in human cell lines may also become contaminated with NGNA that is incorporated from animal-derived culture medium materials [100]. NGNA in cells is recycled in lysosomes and incorporated into glycoproteins [101].

In conclusion, NGNA on Fc-glycans is potentially immunogenic and for this reason is most probably a CQA.

Terminal Sialic acid

The anti-inflammatory activity of immunoglobulin has repeatedly been linked to Fc sialylation, which may lead to decreased ADCC activity and, in some cases, even affect target binding [102-107]. In general, the amount of sialylated Fc glycans of therapeutic antibodies is very low [108].

In conclusion if there is a significant amount of sialylated glycans, and ADCC is part of the MoA of the therapeutic antibody, the influence of sialylation on FcγRIIIa binding and ADCC should be determined *in vitro* as part of the CQA assessment.

α1,6-core fucose

Changes in the levels of afucosylated glycostructures on therapeutic antibodies may be of concern from a safety perspective. The lack of core fucose (i.e. the α1,6-linked fucose on the GlcNAc residue at the reducing terminal of the N-glycosylation site) on the carbohydrate moiety linked to the Fc region of IgG leads to a significantly enhanced *in vitro* ADCC of IgG1 via an increased affinity to FcγRIIIa on immune cells such as natural killer (NK) cells and macrophages [109-117].

Toxicity due to off-target binding may also be a concern for antibodies with higher ADCC. Jiang *et al.* indicate that increased afucosylation leading to increased effector function is a potential safety concern for monoclonal antibodies with moderate or low effector function [22]. Increased crosslinking of activating FcγRs due to higher levels of afucose may also be a safety concern due to the additional release of proinflammatory cytokines such as TNFα and IFNγ.

The lack of core fucose (i.e. the α1,6-linked fucose on the GlcNAc residue involved in the amide bond with the asparagine of the N-glycosylation site) on the carbohydrate moiety linked to the Fc region of an IgG molecule leads to a pronounced increase in ADCC via increased affinity for the FcγRIIIa expressed on immune cells such as NK cells and macrophages [118-126]. Additionally, the absence of core fucose leads to increased Fc-dependent binding to FcγRIII positive non-classical/intermediate monocytes and macrophages, which translates into increased ADCP [127]. In contrast, binding to FcγRI and II-positive and FcγRIII-negative classical monocytes, as well as ADCP mediated by these cells, remain unchanged,

irrespective of fucosylation levels [128].While afucosylation increases ADCP in glycoengineered antibodies, the impact on ADCP due to small variations in afucosylation levels resulting from standard CHO-based manufacturing processes remains to be assessed. Finally, increased afucosylation has not been demonstrated to have any impact on CDC activity. Studies by Junttila et al. highlighted the impact of afucosylation on pharmacological properties and half-life of an IgG1, relative to a wild type control. In these studies, afucosylated trastuzumab displayed moderately altered pharmacokinetic dispositions compared with trastuzumab, slightly faster elimination from the circulation, and a modest reduction in half-life. [129]. The authors hypothesized that the faster clearance may result from the differential bio-distribution that leads to the enrichment of antibodies to immune effector cell-rich organs due to the increased Fc•RIII affinity. In conclusion concerning the scientific literature there is no clear evidence that afucosylated Fc glycans might be of concern for safety. If the afucosylated Fc glycans are outside of the manufacturing range they should be considered from a safety or efficacy perspective. There is clear evidence that afucosylated Fc glycans increase ADCC and if ADCC is part of the mechanism of action, afucosylated Fc-glycans are always likely to be a CQA concerning efficacy.

Bisecting N-acetyl-glucosamine (GlcNAc)

CHO cells lack the gene encoding GnTIII. Therefore antibodies produced in CHO cells lack detectable bisecting GlcNAc glycostructures [130]. GnTIII overexpressed in antibody-producing cells catalyzes the addition of GlcNAc to N-linked oligosaccharides. The addition of bisecting GlcNAc inhibits core-fucosylation and conversion of hybrid to complex glycans [131].

In general, glycostructures containing bisecting GlcNAc are abundant in human polyclonal IgG and are not a considered a safety concern [132]. Recently, Ritamo et al. showed that 7% of the Fc-glycans on intravenous IgG (IVIG) contain glycan structures with bisecting GlcNAc (analyzed at the glycopeptide level) [133]. Therefore, it is unlikely that ATAs are produced against the bisecting GlcNAc epitope. In summary bisecting GlcNAc structures are not a safety issue.

Galactose

Highly galactosylated glycostructures on therapeutic antibodies may be of concern as they increase *in vitro* C1q-binding and CDC activity [134;135]. Increased galactosylation of CHO-derived antibodies has been shown to increase FcγRII and Fc•RIIIa binding as measured using surface plasmon resonance. [136]. Additionally, it has been demonstrated that the increased galactosylation enhances ADCC activity [22]. However, the impact of galactosylation is subtle compared to the impact of afucosylation. [137].

Several reports indicate that a high degree of galactosylation promotes increased binding to C1q leading to enhanced activation of the complement system and CDC activity [138-140]. Similar effects have been observed for the glycoengineered therapeutic antibody obinutuzumab *in vitro,* although CDC is not considered to be a relevant *in vivo* activity.

Several reports state that the extent of terminal galactosylation does not affect ADCC in standard IgG1 antibodies[141-146](Boyd *et al.*, 1995a;Shinkawa *et al.*, 2003e;Hodoniczky *et al.*, 2005d). Another report found that terminal galactosylation has a positive effect on FcγRIIIa binding(Houde et al.,2010). We have

recently demonstrated enhanced ADCC upon enzymatic hypergalactosylation of four different monoclonal antibodies produced using standard CHO manufacturing processes (Thomann et al 2015; Tejada et al 2015). We also showed that elevated levels of terminal galactose have no effect on ADCC of two different glycoengineered therapeutic antibodies (Thomann et al 2015), indicating that the extent to which terminal galactose modulates ADCC depends on the background level of afucosylation. Lastly, we quantitatively compared the effects of galactosylation and afucosylation in the context of glycan heterogeneity to demonstrate that while galactose can influence ADCC activity, afucosylation remains the primary driver of this activity (Thomann et al 2015; Tejada et al 2015).

Karsten *et al.* recently reported an anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIb and dectin-1 [147]. The authors concluded that high N-glycan galactosylation of IgG1 molecules promotes cooperative signaling of the FcγRIIb with dectin-1, resulting in an inhibitory signaling pathway that blocks proinflammatory effector functions.

In conclusion if CDC is part of the MoA for a therapeutic antibody the impact of galactosylation levels reflecting the manufacturing experience should be considered as it relates to efficacy during CQA assessment. If ADCC is part of the MoA of the therapeutic antibody, the impact of galactosylation should be determined by *in vitro* studies as part of CQA assessment

High mannose glycostructures

No data or direct evidence in literature supports the immunogenicity of high mannose glycan structures [148]. Studies by Zhou *et al.* reported enhanced FcγRIIIa binding and ADCC in antibodies with oligomannose-type glycans generated using kifunensine [149]. Kanda *et al.* [150] generated core fucose-lacking human IgG1 antibodies with three different N-linked Fc oligosaccharides, namely, a high-mannose, hybrid, and complex type, using the same producing clone. They demonstrated that the mannosylated glycostructures could induce ADCC via FcγRIIIa binding but to a lesser extent than the hybrid and complex structures. The same holds true for CDC and C1q binding. More recently, Yu *et al.* confirmed that high mannose glycoforms exhibited higher FcγRIIIa binding and ADCC activity as well as decreased C1q binding and CDC activity [151].

In general, core fucose is absent in the highly mannosylated glycoforms generated. Therefore, it remains unclear if the observed increases in FcγRIIIa binding and ADCC activity are due to the presence of multiple mannose moieties, or the absence of core fucose. The reduced C1q binding and CDC activity observed in these studies may also reflect the lack of terminal galactose in these highly mannosylated forms.

Recent studies show clear evidence for selective clearance of oligomannose forms of IgG1 and IgG2 most likely via a mannose receptor-mediated mechanisms [152-154]. Goetze *et al.* used IgG1 and IgG2 in human studies to demonstrate that Man5 isoforms were preferentially removed from circulation, independent of the route of administration [155]. In conclusion if there is a significant amount of mannosylated glycans and if ADCC is part of the mode of action of a therapeutic antibody mannosylation may be a CQA concerning efficacy. Additionally there is clear evidence that oligomannose bearing IgG s are faster cleared than others and therefore may be a CQA concerning PK/PD.

Non-glycosylated heavy chain (NGHC)

Jung et al. concluded that the unmasking of the region around Asn297 does not result in the formation of a neo-epitope [156]. At present, no adverse immunogenicity effects associated with high levels of NGHC have been reported in any clinical study. Furthermore, antibodies with high levels of NGHC have significantly reduced bioactivity and effector function. Therefore, non-glycosylated heavy chain is likely not a concern from a safety or immunogenicity perspective. Aglycosylation of Asn297 completely abolishes binding to FcγRIII and reduces the binding affinity to C1q 10-fold [73;157]. The reduced binding affinity of aglycosylated antibodies abrogates both ADCC and CDC activities [73;158-162]. Ha *et al.* isolated hemi-glycosylated mAb. It was separated from fully glycosylated and aglycosylated forms using cation-exchange chromatography[163].These studies demonstrated that the hemi-glycosylated mAb had decreased binding to all FcγR including both activating and inhibiting receptors. The hemi-glycosylated form also showed decreased ADCC using PBMCs as effector cells. The studies also showed a modest, but statistically significant, decrease in C1q binding for the hemi-glycosylated mAb. Using knob/hole technology to generate heterodimers with different glycan structures, Shatz *et al.* showed that removal of fucose from only one chain of a fully-glycosylated heterodimer is sufficient to fully restore ADCC activity relative to a wild type molecule [164]. However, reduced potencies were observed for hemi-glycosylated or hemi-glycosylated-hemi-afucosylated heterodimers, relative to wild type or a fully-glycosylated heterodimer. These studies showed that afucosylation has a significant impact on the ADCC activity of the hemi-glycosylated species; however, the impact of afucosylation is far greater when both heavy chains are glycosylated. Aglycosylated antibodies have been shown to exhibit conformational changes, decreased thermal stability, increased tendency to aggregate, and loss of effector functions [165;166]. Evidence regarding the impact of aglycosylation on PK is inconclusive as there are studies demonstrating decreased and normal half-life. For example, comparisons of recombinant chimeric or mouse monoclonal antibodies against N297A mutant versions in mouse or rat show reduced half-lives for the mutant aglycosylated antibodies compared to their wild type counterparts [167;168].

In contrast, Hristodorov *et al.* generated six different wild type antibodies and compared them to aglycosylated versions, generated via N297A mutation [169] The aglycosylated antibodies were found to be less stable and displayed a propensity to aggregate when subjected to low pH reflecting viral inactivation conditions common in the antibody manufacturing process. The PK properties of both wild type and aglycosylated antibodies were determined to be nearly identical in rat studies. Tao *et al.* used a chimeric mouse-human IgG and found a shorter half-life for aglycosylated IgG3 compared to that of wild-type IgG3 in mice, while no change was observed for aglycosylated IgG1 compared to wild-type IgG1 [73]. Jung *et al.* state that aglycosylated antibodies display PK that are at least comparable to that of "normal" IgG therapeutics [170].

In conclusion the literature is not quite clear here and if there is a significant amount of non-glycosylated heavy chain the influence on should be assessed by in vitro studies to decide if it is a CQA or not (see also Table 2).

Table 2. Impact of Fc glycans on safety/immunogenicity, biologic activity/efficacy and clearance (PK/PD).

Glycan species	Safety/Immunogenicity	Biologic activity/Efficacy	Clearance (PK/PD)
Galactose	unknown	+	unknown
alpha1,3-Galactose	--	unknown	unknown
Fucose	(-)	++	unknown
bisecting GlcNAc	(-)	+	unknown
Mannose (high-mannose)	unknown	+	--
NANA	unknown	(-)	+
NGNA	--	(-)	+
ß1,2-Xylose/alpha1,3Fukose	--	unknown	unknown
non-glycosylated heavy chain	unknown	--	(-)

+   positive impact   - negativ impact

++   High positive impact   - high negative impact

(+/-)   potential impact

Discussion

The Quality by Design paradigm requires an in-depth understanding of i) the mechanism(s) of action of the biotherapeutic, which are associated with the desired clinical activity, ii) the properties (CQA) of the biotherapeutic that are associated with the desired clinical activity, iii) the process parameters that impact the CQA, and iv) the control of the manufacturing process through a timely analysis and monitoring of the CQA, along with appropriate control of process components such as raw and in-process materials. This ensures the product with the desired qualities is achieved consistently over time.

For monoclonal antibody therapeutics specifically, glycosylation is one of the most relevant post-translational modifications in the manufacturing process. This results in remarkable heterogeneity of the antibody glycoforms. The diverse set of hosts used by industry in the manufacture of monoclonal antibodies also increases this glyco-heterogeneity, which can be critical to antibodies associated with mechanisms of action such as CDC, ADCC and ADCP.

Different glycovariants, such as mannose, have been reported to reduce the serum half-life of therapeutic antibodies while other Fc glycan structural elements such as α1-3 bound galactose and N-glycolylneuraminic acid may be involved in adverse immune reactions. Both should be considered in terms of efficacy and safety, respectively.

The intimate relationship established between Fc glycosylation of the mechanisms associated with the clinical activity of therapeutic antibodies makes the thorough analysis and characterization of the

glycosylation-specific CQA critical to the appropriate assessment of the impact that changes observed in specific glycovariants may have on safety and/or efficacy. This information can appropriately inform manufacturing process development such that these processes are more finely adjusted to deliver the desired Fc glycosylation.

Abbreviations

ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cell-mediated cytotoxicity; AE, adverse events; ATA, anti-therapeutic antibody; CDC, complement-dependent cytotoxicity; CHO, Chinese hamster ovary; CQA, critical quality attribute; C1q, Complement 1q; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; Fab, fragment antigen binding; Fc, fraction crystalizable; FcγR, Fcγ receptors; FcRn, neonatal Fc receptor; GlcNAc, N-acetyl-glucosamine; GnTIII, N-acetylglucosamine-transferase III; IFN, interferon; IgG, immunoglobulin G; IND, investigational new drug; IRR, infusion related reactions; MBL2, mannose-binding lectin 2; mAb, monoclonal antibody; MMR, macrophage mannose receptor; MoA, mechanism/mode of action; NGHC, non-glycosylated heavy chain; NGNA, N-glycolylneuraminic acid; NK, natural killer cells; PK/PD, pharmacokinetics/pharmacodynamics; PBMC, peripheral blood mononuclear cells; QbD, quality by design; QC, quality control; TNF, tumor necrosis factor.

Reference List

[1]   J.V.Ravetch, Fc receptors, Curr.Opin.Immunol. 9 (1997) 121-125.

[2]   J.V.Ravetch, Fc receptors, Curr.Opin.Immunol. 9 (1997) 121-125.

[3]   R.Jefferis, Glycosylation as a strategy to improve antibody-based therapeutics, Nat.Rev.Drug Discov. 8 (2009) 226-234.

[4]   R.Jefferis, Isotype and glycoform selection for antibody therapeutics, Arch.Biochem.Biophys. 526 (2012) 159-166.

[5]   C.Ferrara, P.Brunker, T.Suter, S.Moser, U.Puntener, and P.Umana, Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous beta1, 4-N-acetylglucosaminyltransferase III and Golgi alpha-mannosidase II, Biotechnol.Bioeng. 93 (2006) 851-861.

[6]   K.Masuda, T.Kubota, E.Kaneko, S.Iida, M.Wakitani, Y.Kobayashi-Natsume, A.Kubota, K.Shitara, and K.Nakamura, Enhanced binding affinity for FcgammaRIIIa of fucose-negative antibody is sufficient to induce maximal antibody-dependent cellular cytoxicity, Mol.Immunol. 44 (2007) 3122-3131.

[7]   Y.Mimura, P.R.Ashton, N.Takahashi, D.J.Harvey, and R.Jefferis, Contrasting glycosylation profiles between Fab and Fc of a human IgG protein studied by electrospray ionization mass spectrometry, J.Immunol.Methods 326 (2007) 116-126.

[8]   T.A.Bowden, K.Baruah, C.H.Coles, D.J.Harvey, X.Yu, B.D.Song, D.I.Stuart, A.R.Aricescu, C.N.Scanlan, E.Y.Jones, and M.Crispin, Chemical and structural analysis of an antibody folding intermediate trapped during glycan biosynthesis, J.Am.Chem.Soc. 134 (2012) 17554-17563.

[9]   A.Beck, E.Wagner-Rousset, M.C.Bussat, M.Lokteff, C.Klinguer-Hamour, J.F.Haeuw, L.Goetsch, T.Wurch, D.A.Van, and N.Corvaia, Trends in glycosylation, glycoanalysis and glycoengineering of therapeutic antibodies and Fc-fusion proteins, Curr.Pharm.Biotechnol. 9 (2008) 482-501.

[10] R.Jefferis, Glycosylation of recombinant antibody therapeutics, Biotechnol.Prog. 21 (2005) 11-16.

[11] Y.Durocher and M.Butler, Expression systems for therapeutic glycoprotein production, Curr.Opin.Biotechnol. 20 (2009) 700-707.

[12] R.Jefferis, Glycosylation as a strategy to improve antibody-based therapeutics, Nat.Rev.Drug Discov. 8 (2009) 226-234.

[13] Y.Durocher and M.Butler, Expression systems for therapeutic glycoprotein production, Curr.Opin.Biotechnol. 20 (2009) 700-707.

[14] R.Jefferis, Glycosylation as a strategy to improve antibody-based therapeutics, Nat.Rev.Drug Discov. 8 (2009) 226-234.

[15] R.B.Parekh, R.A.Dwek, B.J.Sutton, D.L.Fernandes, A.Leung, D.Stanworth, T.W.Rademacher, T.Mizuochi, T.Taniguchi, K.Matsuta, and ., Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG, Nature 316 (1985) 452-457.

[16] L.Huang, S.Biolsi, K.R.Bales, and U.Kuchibhotla, Impact of variable domain glycosylation on antibody clearance: an LC/MS characterization, Anal.Biochem. 349 (2006) 197-207.

[17] A.Lim, A.Reed-Bogan, and B.J.Harmon, Glycosylation profiling of a therapeutic recombinant monoclonal antibody with two N-linked glycosylation sites using liquid chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer, Anal.Biochem. 375 (2008) 163-172.

[18] M.Holland, H.Yagi, N.Takahashi, K.Kato, C.O.Savage, D.M.Goodall, and R.Jefferis, 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 (2006) 669-677.

[19] J.Hodoniczky, Y.Z.Zheng, and D.C.James, Control of recombinant monoclonal antibody effector functions by Fc N-glycan remodeling in vitro, Biotechnol.Prog. 21 (2005) 1644-1652.

[20] D.Houde, Y.Peng, S.A.Berkowitz, and J.R.Engen, Post-translational modifications differentially affect IgG1 conformation and receptor binding, Mol.Cell Proteomics. 9 (2010) 1716-1728.

[21] F.Nimmerjahn, R.M.Anthony, and J.V.Ravetch, Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity, Proc.Natl.Acad.Sci.U.S.A 104 (2007) 8433-8437.

[22] X.R.Jiang, A.Song, S.Bergelson, T.Arroll, B.Parekh, K.May, S.Chung, R.Strouse, A.Mire-Sluis, and M.Schenerman, Advances in the assessment and control of the effector functions of therapeutic antibodies, Nat.Rev.Drug Discov. 10 (2011) 101-111.

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

[24] J.Golay, R.F.Da, L.Bologna, C.Ferrara, J.H.Leusen, A.Rambaldi, C.Klein, and M.Introna, Glycoengineered CD20 antibody obinutuzumab activates neutrophils and mediates phagocytosis through CD16B more efficiently than rituximab, Blood 122 (2013) 3482-3491.

[25] S.Herter, M.C.Birk, C.Klein, C.Gerdes, P.Umana, and M.Bacac, Glycoengineering of Therapeutic Antibodies Enhances Monocyte/Macrophage-Mediated Phagocytosis and Cytotoxicity, J.Immunol. (2014).

[26] A.M.Goetze, Y.D.Liu, Z.Zhang, B.Shah, E.Lee, P.V.Bondarenko, and G.C.Flynn, High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans, Glycobiology 21 (2011) 949-959.

[27] M.M.Newkirk, J.Novick, M.M.Stevenson, M.J.Fournier, and P.Apostolakos, Differential clearance of glycoforms of IgG in normal and autoimmune-prone mice, Clin.Exp.Immunol. 106 (1996) 259-264.

[28] C.H.Chung, B.Mirakhur, E.Chan, Q.T.Le, J.Berlin, M.Morse, B.A.Murphy, S.M.Satinover, J.Hosen, D.Mauro, R.J.Slebos, Q.Zhou, D.Gold, T.Hatley, D.J.Hicklin, and T.A.Platts-Mills, Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose, N.Engl.J.Med. 358 (2008) 1109-1117.

[29] A.Zhu and R.Hurst, Anti-N-glycolylneuraminic acid antibodies identified in healthy human serum, Xenotransplantation. 9 (2002) 376-381.

[30] F.Bibeau, E.Lopez-Crapez, F.F.Di, S.Thezenas, M.Ychou, F.Blanchard, A.Lamy, F.Penault-Llorca, T.Frebourg, P.Michel, J.C.Sabourin, and F.Boissiere-Michot, Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan, J.Clin.Oncol. 27 (2009) 1122-1129.

[31] G.Cartron, L.Dacheux, G.Salles, P.Solal-Celigny, P.Bardos, P.Colombat, and H.Watier, Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene, Blood 99 (2002) 754-758.

[32] A.Musolino, N.Naldi, B.Bortesi, D.Pezzuolo, M.Capelletti, G.Missale, D.Laccabue, A.Zerbini, R.Camisa, G.Bisagni, T.M.Neri, and A.Ardizzoni, Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer, J.Clin.Oncol. 26 (2008) 1789-1796.

[33] A.Beck, E.Wagner-Rousset, M.C.Bussat, M.Lokteff, C.Klinguer-Hamour, J.F.Haeuw, L.Goetsch, T.Wurch, D.A.Van, and N.Corvaia, Trends in glycosylation, glycoanalysis and glycoengineering of therapeutic antibodies and Fc-fusion proteins, Curr.Pharm.Biotechnol. 9 (2008) 482-501.

[34] T.Shinkawa, K.Nakamura, N.Yamane, E.Shoji-Hosaka, Y.Kanda, M.Sakurada, K.Uchida, H.Anazawa, M.Satoh, M.Yamasaki, N.Hanai, and K.Shitara, The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity, J.Biol.Chem. 278 (2003) 3466-3473.

[35] C.Ferrara, P.Brunker, T.Suter, S.Moser, U.Puntener, and P.Umana, Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous beta1, 4-N-acetylglucosaminyltransferase III and Golgi alpha-mannosidase II, Biotechnol.Bioeng. 93 (2006) 851-861.

[36] S.Matsumiya, Y.Yamaguchi, J.Saito, M.Nagano, H.Sasakawa, S.Otaki, M.Satoh, K.Shitara, and K.Kato, Structural comparison of fucosylated and nonfucosylated Fc fragments of human immunoglobulin G1, J.Mol.Biol. 368 (2007) 767-779.

[37] K.Mori, R.Kuni-Kamochi, N.Yamane-Ohnuki, M.Wakitani, K.Yamano, H.Imai, Y.Kanda, R.Niwa, S.Iida, K.Uchida, K.Shitara, and M.Satoh, Engineering Chinese hamster ovary cells to maximize effector function of produced antibodies using FUT8 siRNA, Biotechnol.Bioeng. 88 (2004) 901-908.

[38] T.Shinkawa, K.Nakamura, N.Yamane, E.Shoji-Hosaka, Y.Kanda, M.Sakurada, K.Uchida, H.Anazawa, M.Satoh, M.Yamasaki, N.Hanai, and K.Shitara, The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity, J.Biol.Chem. 278 (2003) 3466-3473.

[39] T.Shinkawa, K.Nakamura, N.Yamane, E.Shoji-Hosaka, Y.Kanda, M.Sakurada, K.Uchida, H.Anazawa, M.Satoh, M.Yamasaki, N.Hanai, and K.Shitara, The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity, J.Biol.Chem. 278 (2003) 3466-3473.

[40] D.Reusch, M.Haberger, B.Maier, M.Maier, R.Kloseck, B.Zimmermann, M.Hook, Z.Szabo, S.Tep, J.Wegstein, N.Alt, P.Bulau, and M.Wuhrer, Comparison of methods for the analysis of therapeutic immunoglobulin G Fc-glycosylation profiles--part 1: separation-based methods, MAbs. 7 (2015) 167-179.

[41] B.A.Baldo, Adverse events to monoclonal antibodies used for cancer therapy: Focus on hypersensitivity responses, Oncoimmunology. 2 (2013) e26333.

[42] M.Daeron, Fc receptor biology, Annu.Rev.Immunol. 15 (1997) 203-234.

[43] J.S.Gerber and D.M.Mosser, Stimulatory and inhibitory signals originating from the macrophage Fcgamma receptors, Microbes.Infect. 3 (2001) 131-139.

[44] J.Lund, N.Takahashi, J.D.Pound, M.Goodall, H.Nakagawa, and R.Jefferis, Oligosaccharide-protein interactions in IgG can modulate recognition by Fc gamma receptors, FASEB J. 9 (1995) 115-119.

[45] F.Nimmerjahn and J.V.Ravetch, Fcgamma receptors: old friends and new family members, Immunity. 24 (2006) 19-28.

[46] S.Radaev and P.Sun, Recognition of immunoglobulins by Fcgamma receptors, Mol.Immunol. 38 (2002) 1073-1083.

[47] J.V.Ravetch, Fc receptors, Curr.Opin.Immunol. 9 (1997) 121-125.

[48] J.V.Ravetch and S.Bolland, IgG Fc receptors, Annu.Rev.Immunol. 19 (2001) 275-290.

[49] P.Sondermann, J.Kaiser, and U.Jacob, Molecular basis for immune complex recognition: a comparison of Fc-receptor structures, J.Mol.Biol. 309 (2001) 737-749.

[50] R.Jefferis, Isotype and glycoform selection for antibody therapeutics, Arch.Biochem.Biophys. 526 (2012) 159-166.

[51] V.Ghetie and E.S.Ward, Multiple roles for the major histocompatibility complex class I- related receptor FcRn, Annu.Rev.Immunol. 18 (2000) 739-766.

[52] D.C.Roopenian and S.Akilesh, FcRn: the neonatal Fc receptor comes of age, Nat.Rev.Immunol. 7 (2007) 715-725.

[53] S.Akilesh, G.J.Christianson, D.C.Roopenian, and A.S.Shaw, Neonatal FcR expression in bone marrow-derived cells functions to protect serum IgG from catabolism, J.Immunol. 179 (2007) 4580-4588.

[54] T.T.Kuo, E.J.de Muinck, S.M.Claypool, M.Yoshida, T.Nagaishi, V.G.Aveson, W.I.Lencer, and R.S.Blumberg, N-Glycan Moieties in Neonatal Fc Receptor Determine Steady-state Membrane Distribution and Directional Transport of IgG, J.Biol.Chem. 284 (2009) 8292-8300.

[55] D.B.Tesar and P.J.Bjorkman, An intracellular traffic jam: Fc receptor-mediated transport of immunoglobulin G, Curr.Opin.Struct.Biol. 20 (2010) 226-233.

[56] T.E.Wileman, M.R.Lennartz, and P.D.Stahl, Identification of the macrophage mannose receptor as a 175-kDa membrane protein, Proc.Natl.Acad.Sci.U.S.A 83 (1986) 2501-2505.

[57] P.Allavena, M.Chieppa, P.Monti, and L.Piemonti, From pattern recognition receptor to regulator of homeostasis: the double-faced macrophage mannose receptor, Crit Rev.Immunol. 24 (2004) 179-192.

[58] X.Dong, W.J.Storkus, and R.D.Salter, Binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells, J.Immunol. 163 (1999) 5427-5434.

[59] W.I.Weis, M.E.Taylor, and K.Drickamer, The C-type lectin superfamily in the immune system, Immunol.Rev. 163 (1998) 19-34.

[60] S.J.Lee, S.Evers, D.Roeder, A.F.Parlow, J.Risteli, L.Risteli, Y.C.Lee, T.Feizi, H.Langen, and M.C.Nussenzweig, Mannose receptor-mediated regulation of serum glycoprotein homeostasis, Science 295 (2002) 1898-1901.

[61] R.Deng, F.Jin, S.Prabhu, and S.Iyer, Monoclonal antibodies: what are the pharmacokinetic and pharmacodynamic considerations for drug development?, Expert.Opin.Drug Metab Toxicol. 8 (2012) 141-160.

[62] X.Chen, Y.D.Liu, and G.C.Flynn, The effect of Fc glycan forms on human IgG2 antibody clearance in humans, Glycobiology 19 (2009) 240-249.

[63] R.J.Harris, Heterogeneity of recombinant antibodies: linking structure to function, Dev.Biol.(Basel) 122 (2005) 117-127.

[64] L.Huang, S.Biolsi, K.R.Bales, and U.Kuchibhotla, Impact of variable domain glycosylation on antibody clearance: an LC/MS characterization, Anal.Biochem. 349 (2006) 197-207.

[65] A.J.Jones, D.I.Papac, E.H.Chin, R.Keck, S.A.Baughman, Y.S.Lin, J.Kneer, and J.E.Battersby, Selective clearance of glycoforms of a complex glycoprotein pharmaceutical caused by terminal N-acetylglucosamine is similar in humans and cynomolgus monkeys, Glycobiology 17 (2007) 529-540.

[66] T.A.Millward, M.Heitzmann, K.Bill, U.Langle, P.Schumacher, and K.Forrer, Effect of constant and variable domain glycosylation on pharmacokinetics of therapeutic antibodies in mice, Biologicals 36 (2008) 41-47.

[67] Y.Kanda, T.Yamada, K.Mori, A.Okazaki, M.Inoue, K.Kitajima-Miyama, R.Kuni-Kamochi, R.Nakano, K.Yano, S.Kakita, K.Shitara, and M.Satoh, 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 (2007) 104-118.

[68] M.M.Newkirk, J.Novick, M.M.Stevenson, M.J.Fournier, and P.Apostolakos, Differential clearance of glycoforms of IgG in normal and autoimmune-prone mice, Clin.Exp.Immunol. 106 (1996) 259-264.

[69] A.Wright and S.L.Morrison, Effect of altered CH2-associated carbohydrate structure on the functional properties and in vivo fate of chimeric mouse-human immunoglobulin G1, J.Exp.Med. 180 (1994) 1087-1096.

[70] M.K.Leabman, Y.G.Meng, R.F.Kelley, L.E.DeForge, K.J.Cowan, and S.Iyer, Effects of altered FcgammaR binding on antibody pharmacokinetics in cynomolgus monkeys, MAbs. 5 (2013) 896-903.

[71] A.J.Jones, D.I.Papac, E.H.Chin, R.Keck, S.A.Baughman, Y.S.Lin, J.Kneer, and J.E.Battersby, Selective clearance of glycoforms of a complex glycoprotein pharmaceutical caused by terminal N-acetylglucosamine is similar in humans and cynomolgus monkeys, Glycobiology 17 (2007) 529-540.

[72] R.Keck, N.Nayak, L.Lerner, S.Raju, S.Ma, T.Schreitmueller, S.Chamow, K.Moorhouse, C.Kotts, and A.Jones, Characterization of a complex glycoprotein whose variable metabolic clearance in humans is dependent on terminal N-acetylglucosamine content, Biologicals 36 (2008) 49-60.

[73] M.H.Tao and S.L.Morrison, 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 (1989) 2595-2601.

[74] X.Dong, W.J.Storkus, and R.D.Salter, Binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells, J.Immunol. 163 (1999) 5427-5434.

[75] U.Galili, E.A.Rachmilewitz, A.Peleg, and I.Flechner, A unique natural human IgG antibody with anti-alpha-galactosyl specificity, J.Exp.Med. 160 (1984) 1519-1531.

[76] C.H.Chung, B.Mirakhur, E.Chan, Q.T.Le, J.Berlin, M.Morse, B.A.Murphy, S.M.Satinover, J.Hosen, D.Mauro, R.J.Slebos, Q.Zhou, D.Gold, T.Hatley, D.J.Hicklin, and T.A.Platts-Mills, Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose, N.Engl.J.Med. 358 (2008) 1109-1117.

[77] R.D.Larsen, V.P.Rajan, M.M.Ruff, J.Kukowska-Latallo, R.D.Cummings, and J.B.Lowe, Isolation of a cDNA encoding a murine UDPgalactose:beta-D-galactosyl- 1,4-N-acetyl-D-glucosaminide alpha-1,3-galactosyltransferase: expression cloning by gene transfer, Proc.Natl.Acad.Sci.U.S.A 86 (1989) 8227-8231.

[78] D.M.Sheeley, B.M.Merrill, and L.C.Taylor, Characterization of monoclonal antibody glycosylation: comparison of expression systems and identification of terminal alpha-linked galactose, Anal.Biochem. 247 (1997) 102-110.

[79] N.Jenkins, R.B.Parekh, and D.C.James, Getting the glycosylation right: implications for the biotechnology industry, Nat.Biotechnol. 14 (1996) 975-981.

[80] C.J.Bosques, B.E.Collins, J.W.Meador, III, H.Sarvaiya, J.L.Murphy, G.Dellorusso, D.A.Bulik, I.H.Hsu, N.Washburn, S.F.Sipsey, J.R.Myette, R.Raman, Z.Shriver, R.Sasisekharan, and G.Venkataraman, Chinese hamster ovary cells can produce galactose-alpha-1,3-galactose antigens on proteins, Nat.Biotechnol. 28 (2010) 1153-1156.

[81] U.Galili, The alpha-Gal epitope (Galalpha1-3Galbeta1-4GlcNAc-R) in xenotransplantation, Biochimie 83 (2001) 557-563.

[82] C.K.Borrebaeck, A.C.Malmborg, and M.Ohlin, Does endogenous glycosylation prevent the use of mouse monoclonal antibodies as cancer therapeutics?, Immunol.Today 14 (1993) 477-479.

[83] N.Deglon, V.Aubert, F.Spertini, L.Winkel, and P.Aebischer, Presence of Gal-alpha1,3Gal epitope on xenogeneic lines: implications for cellular gene therapy based on the encapsulation technology, Xenotransplantation. 10 (2003) 204-213.

[84] J.Qian, T.Liu, L.Yang, A.Daus, R.Crowley, and Q.Zhou, 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 (2007) 8-18.

[85] C.H.Chung, B.Mirakhur, E.Chan, Q.T.Le, J.Berlin, M.Morse, B.A.Murphy, S.M.Satinover, J.Hosen, D.Mauro, R.J.Slebos, Q.Zhou, D.Gold, T.Hatley, D.J.Hicklin, and T.A.Platts-Mills, Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose, N.Engl.J.Med. 358 (2008) 1109-1117.

[86] J.J.Lammerts van Bueren, T.Rispens, S.Verploegen, d.P.-M.van, S.Stapel, L.J.Workman, H.James, P.H.van Berkel, J.G.van de Winkel, T.A.Platts-Mills, and P.W.Parren, Anti-galactose-alpha-1,3-galactose IgE from allergic patients does not bind alpha-galactosylated glycans on intact therapeutic antibody Fc domains, Nat.Biotechnol. 29 (2011) 574-576.

[87] Y.Tekoah, K.Ko, H.Koprowski, D.J.Harvey, M.R.Wormald, R.A.Dwek, and P.M.Rudd, Controlled glycosylation of therapeutic antibodies in plants, Arch.Biochem.Biophys. 426 (2004) 266-278.

[88] K.E.Paulus, V.Mahler, M.Pabst, K.H.Kogel, F.Altmann, and U.Sonnewald, Silencing beta1,2-xylosyltransferase in Transgenic Tomato Fruits Reveals xylose as Constitutive Component of Ige-Binding Epitopes, Front Plant Sci. 2 (2011) 42.

[89] R.Strasser, F.Altmann, L.Mach, J.Glossl, and H.Steinkellner, Generation of Arabidopsis thaliana plants with complex N-glycans lacking beta1,2-linked xylose and core alpha1,3-linked fucose, FEBS Lett. 561 (2004) 132-136.

[90] C.H.Hokke, A.A.Bergwerff, G.W.van Dedem, O.J.van, J.P.Kamerling, and J.F.Vliegenthart, Sialylated carbohydrate chains of recombinant human glycoproteins expressed in Chinese hamster ovary cells contain traces of N-glycolylneuraminic acid, FEBS Lett. 275 (1990) 9-14.

[91] A.Noguchi, C.J.Mukuria, E.Suzuki, and M.Naiki, Failure of human immunoresponse to N-glycolylneuraminic acid epitope contained in recombinant human erythropoietin, Nephron 72 (1996) 599-603.

[92] A.Varki, Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins, Nature 446 (2007) 1023-1029.

[93] P.Tangvoranuntakul, P.Gagneux, S.Diaz, M.Bardor, N.Varki, A.Varki, and E.Muchmore, Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid, Proc.Natl.Acad.Sci.U.S.A 100 (2003) 12045-12050.

[94] V.Padler-Karavani, H.Yu, H.Cao, H.Chokhawala, F.Karp, N.Varki, X.Chen, and A.Varki, Diversity in specificity, abundance, and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease, Glycobiology 18 (2008) 818-830.

[95] V.Padler-Karavani, H.Yu, H.Cao, H.Chokhawala, F.Karp, N.Varki, X.Chen, and A.Varki, Diversity in specificity, abundance, and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease, Glycobiology 18 (2008) 818-830.

[96] A.Zhu and R.Hurst, Anti-N-glycolylneuraminic acid antibodies identified in healthy human serum, Xenotransplantation. 9 (2002) 376-381.

[97] Y.Durocher and M.Butler, Expression systems for therapeutic glycoprotein production, Curr.Opin.Biotechnol. 20 (2009) 700-707.

[98] J.Milland and M.S.Sandrin, ABO blood group and related antigens, natural antibodies and transplantation, Tissue Antigens 68 (2006) 459-466.

[99] E.Higgins, Carbohydrate analysis throughout the development of a protein therapeutic, Glycoconj.J. 27 (2010) 211-225.

[100] D.Ghaderi, R.E.Taylor, V.Padler-Karavani, S.Diaz, and A.Varki, Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins, Nat.Biotechnol. 28 (2010) 863-867.

[101] M.Bardor, D.H.Nguyen, S.Diaz, and A.Varki, Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells, J.Biol.Chem. 280 (2005) 4228-4237.

[102] Y.Kaneko, F.Nimmerjahn, and J.V.Ravetch, Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation, Science 313 (2006) 670-673.

[103] F.Nimmerjahn and J.V.Ravetch, Anti-inflammatory actions of intravenous immunoglobulin, Annu.Rev.Immunol. 26 (2008) 513-533.

[104] K.Fukushima and S.Takasaki, Suppressive role of sialylated N-glycans in Fc receptor-mediated phagocytosis by macrophages, Biochem.Biophys.Res.Commun. 192 (1993) 333-337.

[105] R.M.Anthony, F.Nimmerjahn, D.J.Ashline, V.N.Reinhold, J.C.Paulson, and J.V.Ravetch, Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc, Science 320 (2008) 373-376.

[106] R.M.Anthony, F.Wermeling, M.C.Karlsson, and J.V.Ravetch, Identification of a receptor required for the anti-inflammatory activity of IVIG, Proc.Natl.Acad.Sci.U.S.A 105 (2008) 19571-19578.

[107] B.J.Scallon, S.H.Tam, S.G.McCarthy, A.N.Cai, and T.S.Raju, Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality, Mol.Immunol. 44 (2007) 1524-1534.

[108] R.Jefferis, A sugar switch for anti-inflammatory antibodies, Nat.Biotechnol. 24 (2006) 1230-1231.

[109] S.Chung, V.Quarmby, X.Gao, Y.Ying, L.Lin, C.Reed, C.Fong, W.Lau, Z.J.Qiu, A.Shen, M.Vanderlaan, and A.Song, Quantitative evaluation of fucose reducing effects in a humanized antibody on Fcgamma receptor binding and antibody-dependent cell-mediated cytotoxicity activities, MAbs. 4 (2012).

[110] S.Iida, R.Kuni-Kamochi, K.Mori, H.Misaka, M.Inoue, A.Okazaki, K.Shitara, and M.Satoh, Two mechanisms of the enhanced antibody-dependent cellular cytotoxicity (ADCC) efficacy of non-fucosylated therapeutic antibodies in human blood, BMC.Cancer 9 (2009) -58.

[111] R.Jefferis, Antibody therapeutics: isotype and glycoform selection, Expert.Opin.Biol.Ther. 7 (2007) 1401-1413.

[112] R.Niwa, S.Hatanaka, E.Shoji-Hosaka, M.Sakurada, Y.Kobayashi, A.Uehara, H.Yokoi, K.Nakamura, and K.Shitara, Enhancement of the antibody-dependent cellular cytotoxicity of low-fucose IgG1 Is independent of FcgammaRIIIa functional polymorphism, Clin.Cancer Res. 10 (2004) 6248-6255.

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

[114] M.Peipp, J.J.Lammerts van Bueren, T.Schneider-Merck, W.W.Bleeker, M.Dechant, T.Beyer, R.Repp, P.H.van Berkel, T.Vink, J.G.van de Winkel, P.W.Parren, and T.Valerius, Antibody fucosylation differentially impacts cytotoxicity mediated by NK and PMN effector cells, Blood 112 (2008) 2390-2399.

[115] R.L.Shields, J.Lai, R.Keck, L.Y.O'Connell, K.Hong, Y.G.Meng, S.H.Weikert, and L.G.Presta, Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity, J.Biol.Chem. 277 (2002) 26733-26740.

[116] P.Umana, J.Jean-Mairet, R.Moudry, H.Amstutz, and J.E.Bailey, Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity, Nat.Biotechnol. 17 (1999) 176-180.

[117] N.Yamane-Ohnuki, S.Kinoshita, M.Inoue-Urakubo, M.Kusunoki, S.Iida, R.Nakano, M.Wakitani, R.Niwa, M.Sakurada, K.Uchida, K.Shitara, and M.Satoh, 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 (2004) 614-622.

[118] S.Chung, V.Quarmby, X.Gao, Y.Ying, L.Lin, C.Reed, C.Fong, W.Lau, Z.J.Qiu, A.Shen, M.Vanderlaan, and A.Song, Quantitative evaluation of fucose reducing effects in a humanized antibody on Fcgamma receptor binding and antibody-dependent cell-mediated cytotoxicity activities, MAbs. 4 (2012).

[119] R.Niwa, S.Hatanaka, E.Shoji-Hosaka, M.Sakurada, Y.Kobayashi, A.Uehara, H.Yokoi, K.Nakamura, and K.Shitara, Enhancement of the antibody-dependent cellular cytotoxicity of low-fucose IgG1 Is independent of FcgammaRIIIa functional polymorphism, Clin.Cancer Res. 10 (2004) 6248-6255.

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

[121] M.Peipp, J.J.Lammerts van Bueren, T.Schneider-Merck, W.W.Bleeker, M.Dechant, T.Beyer, R.Repp, P.H.van Berkel, T.Vink, J.G.van de Winkel, P.W.Parren, and T.Valerius, Antibody fucosylation differentially impacts cytotoxicity mediated by NK and PMN effector cells, Blood 112 (2008) 2390-2399.

[122] R.L.Shields, J.Lai, R.Keck, L.Y.O'Connell, K.Hong, Y.G.Meng, S.H.Weikert, and L.G.Presta, Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity, J.Biol.Chem. 277 (2002) 26733-26740.

[123] P.Umana, J.Jean-Mairet, R.Moudry, H.Amstutz, and J.E.Bailey, Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity, Nat.Biotechnol. 17 (1999) 176-180.

[124] N.Yamane-Ohnuki, S.Kinoshita, M.Inoue-Urakubo, M.Kusunoki, S.Iida, R.Nakano, M.Wakitani, R.Niwa, M.Sakurada, K.Uchida, K.Shitara, and M.Satoh, 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 (2004) 614-622.

[125] R.L.Shields, J.Lai, R.Keck, L.Y.O'Connell, K.Hong, Y.G.Meng, S.H.Weikert, and L.G.Presta, Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity, J.Biol.Chem. 277 (2002) 26733-26740.

[126] R.J.Rothman, B.Perussia, D.Herlyn, and L.Warren, Antibody-dependent cytotoxicity mediated by natural killer cells is enhanced by castanospermine-induced alterations of IgG glycosylation, Mol.Immunol. 26 (1989) 1113-1123.

[127] S.Herter, M.C.Birk, C.Klein, C.Gerdes, P.Umana, and M.Bacac, Glycoengineering of Therapeutic Antibodies Enhances Monocyte/Macrophage-Mediated Phagocytosis and Cytotoxicity, J.Immunol. (2014).

[128] S.Herter, M.C.Birk, C.Klein, C.Gerdes, P.Umana, and M.Bacac, Glycoengineering of Therapeutic Antibodies Enhances Monocyte/Macrophage-Mediated Phagocytosis and Cytotoxicity, J.Immunol. (2014).

[129] T.T.Junttila, K.Parsons, C.Olsson, Y.Lu, Y.Xin, J.Theriault, L.Crocker, O.Pabonan, T.Baginski, G.Meng, K.Totpal, R.F.Kelley, and M.X.Sliwkowski, Superior in vivo efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer, Cancer Res. 70 (2010) 4481-4489.

[130] Y.Durocher and M.Butler, Expression systems for therapeutic glycoprotein production, Curr.Opin.Biotechnol. 20 (2009) 700-707.

[131] C.Ferrara, P.Brunker, T.Suter, S.Moser, U.Puntener, and P.Umana, Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous beta1, 4-N-acetylglucosaminyltransferase III and Golgi alpha-mannosidase II, Biotechnol.Bioeng. 93 (2006) 851-861.

[132] R.Jefferis, Glycosylation as a strategy to improve antibody-based therapeutics, Nat.Rev.Drug Discov. 8 (2009) 226-234.

[133] I.Ritamo, M.Cloutier, L.Valmu, S.Neron, and J.Rabina, Comparison of the glycosylation of in vitro generated polyclonal human IgG and therapeutic immunoglobulins, Mol.Immunol. 57 (2013) 255-262.

[134] P.N.Boyd, A.C.Lines, and A.K.Patel, The effect of the removal of sialic acid, galactose and total carbohydrate on the functional activity of Campath-1H, Mol.Immunol. 32 (1995) 1311-1318.

[135] N.Tsuchiya, T.Endo, K.Matsuta, S.Yoshinoya, T.Aikawa, E.Kosuge, F.Takeuchi, T.Miyamoto, and A.Kobata, Effects of galactose depletion from oligosaccharide chains on immunological activities of human IgG, J.Rheumatol. 16 (1989) 285-290.

[136] I.Ritamo, M.Cloutier, L.Valmu, S.Neron, and J.Rabina, Comparison of the glycosylation of in vitro generated polyclonal human IgG and therapeutic immunoglobulins, Mol.Immunol. 57 (2013) 255-262.

[137] I.Ritamo, M.Cloutier, L.Valmu, S.Neron, and J.Rabina, Comparison of the glycosylation of in vitro generated polyclonal human IgG and therapeutic immunoglobulins, Mol.Immunol. 57 (2013) 255-262.

[138] J.Hodoniczky, Y.Z.Zheng, and D.C.James, Control of recombinant monoclonal antibody effector functions by Fc N-glycan remodeling in vitro, Biotechnol.Prog. 21 (2005) 1644-1652.

[139] R.Jefferis, Glycosylation as a strategy to improve antibody-based therapeutics, Nat.Rev.Drug Discov. 8 (2009) 226-234.

[140] F.Nimmerjahn, R.M.Anthony, and J.V.Ravetch, Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity, Proc.Natl.Acad.Sci.U.S.A 104 (2007) 8433-8437.

[141] P.N.Boyd, P.N.Boyd, A.C.Lines, A.C.Lines, A.K.Patel, and A.K.Patel, The effect of the removal of sialic acid, galactose and total carbohydrate on the functional activity of Campath-1H, Mol.Immunol. 32 (1995) 1311-1318.

[142] T.Shinkawa, T.Shinkawa, K.Nakamura, K.Nakamura, N.Yamane, N.Yamane, E.Shoji-Hosaka, E.Shoji-Hosaka, Y.Kanda, Y.Kanda, M.Sakurada, M.Sakurada, K.Uchida, K.Uchida, H.Anazawa, H.Anazawa, M.Satoh, M.Satoh, M.Yamasaki, M.Yamasaki, N.Hanai, N.Hanai, K.Shitara, and K.Shitara, The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides

2

shows the critical role of enhancing antibody-dependent cellular cytotoxicity, J.Biol.Chem. 278 (2003) 3466-3473.

[143] J.Hodoniczky, J.Hodoniczky, Y.Z.Zheng, Y.Z.Zheng, D.C.James, and D.C.James, Control of recombinant monoclonal antibody effector functions by Fc N-glycan remodeling in vitro, Biotechnol.Prog. 21 (2005) 1644-1652.

[144] P.N.Boyd, A.C.Lines, and A.K.Patel, The effect of the removal of sialic acid, galactose and total carbohydrate on the functional activity of Campath-1H, Mol.Immunol. 32 (1995) 1311-1318.

[145] J.Hodoniczky, Y.Z.Zheng, and D.C.James, Control of recombinant monoclonal antibody effector functions by Fc N-glycan remodeling in vitro, Biotechnol.Prog. 21 (2005) 1644-1652.

[146] T.Shinkawa, K.Nakamura, N.Yamane, E.Shoji-Hosaka, Y.Kanda, M.Sakurada, K.Uchida, H.Anazawa, M.Satoh, M.Yamasaki, N.Hanai, and K.Shitara, The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity, J.Biol.Chem. 278 (2003) 3466-3473.

[147] C.M.Karsten, M.K.Pandey, J.Figge, R.Kilchenstein, P.R.Taylor, M.Rosas, J.U.McDonald, S.J.Orr, M.Berger, D.Petzold, V.Blanchard, A.Winkler, C.Hess, D.M.Reid, I.V.Majoul, R.T.Strait, N.L.Harris, G.Kohl, E.Wex, R.Ludwig, D.Zillikens, F.Nimmerjahn, F.D.Finkelman, G.D.Brown, M.Ehlers, and J.Kohl, Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcgammaRIIB and dectin-1, Nat.Med. 18 (2012) 1401-1406.

[148] E.Pacis, M.Yu, J.Autsen, R.Bayer, and F.Li, Effects of cell culture conditions on antibody N-linked glycosylation-what affects high mannose 5 glycoform, Biotechnol.Bioeng. (2011).

[149] M.Yu, D.Brown, C.Reed, S.Chung, J.Lutman, E.Stefanich, A.Wong, J.P.Stephan, and R.Bayer, Production, characterization, and pharmacokinetic properties of antibodies with N-linked Mannose-5 glycans, MAbs. 4 (2012) 475-487.

[150] Y.Kanda, T.Yamada, K.Mori, A.Okazaki, M.Inoue, K.Kitajima-Miyama, R.Kuni-Kamochi, R.Nakano, K.Yano, S.Kakita, K.Shitara, and M.Satoh, 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 (2007) 104-118.

[151] M.Yu, D.Brown, C.Reed, S.Chung, J.Lutman, E.Stefanich, A.Wong, J.P.Stephan, and R.Bayer, Production, characterization, and pharmacokinetic properties of antibodies with N-linked Mannose-5 glycans, MAbs. 4 (2012) 475-487.

[152] L.Alessandri, D.Ouellette, A.Acquah, M.Rieser, D.Leblond, M.Saltarelli, C.Radziejewski, T.Fujimori, and I.Correia, Increased serum clearance of oligomannose species present on a human IgG1 molecule, MAbs. 4 (2012) 509-520.

[153] A.M.Goetze, Y.D.Liu, Z.Zhang, B.Shah, E.Lee, P.V.Bondarenko, and G.C.Flynn, High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans, Glycobiology 21 (2011) 949-959.

[154] M.Yu, D.Brown, C.Reed, S.Chung, J.Lutman, E.Stefanich, A.Wong, J.P.Stephan, and R.Bayer, Production, characterization, and pharmacokinetic properties of antibodies with N-linked Mannose-5 glycans, MAbs. 4 (2012) 475-487.

[155] A.M.Goetze, Y.D.Liu, Z.Zhang, B.Shah, E.Lee, P.V.Bondarenko, and G.C.Flynn, High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans, Glycobiology 21 (2011) 949-959.

[156] S.T.Jung, T.H.Kang, W.Kelton, and G.Georgiou, Bypassing glycosylation: engineering aglycosylated full-length IgG antibodies for human therapy, Curr.Opin.Biotechnol. 22 (2011) 858-867.

[157] Y.Mimura, S.Church, R.Ghirlando, P.R.Ashton, S.Dong, M.Goodall, J.Lund, and R.Jefferis, 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 (2000) 697-706.

[158] S.L.Sazinsky, R.G.Ott, N.W.Silver, B.Tidor, J.V.Ravetch, and K.D.Wittrup, Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors, Proc.Natl.Acad.Sci.U.S.A 105 (2008) 20167-20172.

[159] J.Lund, N.Takahashi, J.D.Pound, M.Goodall, and R.Jefferis, Multiple interactions of IgG with its core oligosaccharide can modulate recognition by complement and human Fc gamma receptor I and influence the synthesis of its oligosaccharide chains, J.Immunol. 157 (1996) 4963-4969.

[160] S.T.Jung, S.T.Reddy, T.H.Kang, M.J.Borrok, I.Sandlie, P.W.Tucker, and G.Georgiou, Aglycosylated IgG variants expressed in bacteria that selectively bind FcgammaRI potentiate tumor cell killing by monocyte-dendritic cells, Proc.Natl.Acad.Sci.U.S.A 107 (2010) 604-609.

[161] S.T.Jung, T.H.Kang, W.Kelton, and G.Georgiou, Bypassing glycosylation: engineering aglycosylated full-length IgG antibodies for human therapy, Curr.Opin.Biotechnol. 22 (2011) 858-867.

[162] R.Jefferis, Recombinant antibody therapeutics: the impact of glycosylation on mechanisms of action, Trends Pharmacol.Sci. 30 (2009) 356-362.

[163] S.Ha, Y.Ou, J.Vlasak, Y.Li, S.Wang, K.Vo, Y.Du, A.Mach, Y.Fang, and N.Zhang, Isolation and characterization of IgG1 with asymmetrical Fc glycosylation, Glycobiology 21 (2011) 1087-1096.

[164] W.Shatz, S.Chung, B.Li, B.Marshall, M.Tejada, W.Phung, W.Sandoval, R.F.Kelley, and J.M.Scheer, Knobs-into-holes antibody production in mammalian cell lines reveals that asymmetric afucosylation is sufficient for full antibody-dependent cellular cytotoxicity, MAbs. 5 (2013) 872-881.

[165] R.Ghirlando, J.Lund, M.Goodall, and R.Jefferis, Glycosylation of human IgG-Fc: influences on structure revealed by differential scanning micro-calorimetry, Immunol.Lett. 68 (1999) 47-52.

[166] D.Hristodorov, R.Fischer, H.Joerissen, B.Muller-Tiemann, H.Apeler, and L.Linden, Generation and comparative characterization of glycosylated and aglycosylated human IgG1 antibodies, Mol.Biotechnol. 53 (2013) 326-335.

[167] E.J.Wawrzynczak, G.D.Parnell, A.J.Cumber, P.T.Jones, and G.Winter, Blood clearance in the mouse of an aglycosyl recombinant monoclonal antibody, Biochem.Soc.Trans. 17 (1989) 1061-1062.

[168] E.J.Wawrzynczak, A.J.Cumber, G.D.Parnell, P.T.Jones, and G.Winter, Blood clearance in the rat of a recombinant mouse monoclonal antibody lacking the N-linked oligosaccharide side chains of the CH2 domains, Mol.Immunol. 29 (1992) 213-220.

[169] D.Hristodorov, R.Fischer, H.Joerissen, B.Muller-Tiemann, H.Apeler, and L.Linden, Generation and comparative characterization of glycosylated and aglycosylated human IgG1 antibodies, Mol.Biotechnol. 53 (2013) 326-335.

[170] S.T.Jung, T.H.Kang, W.Kelton, and G.Georgiou, Bypassing glycosylation: engineering aglycosylated full-length IgG antibodies for human therapy, Curr.Opin.Biotechnol. 22 (2011) 858-867.