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Review

IgG-Fc glycoengineering in non-mammalian expression hosts

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ABSTRACT

The remarkable success of therapeutic applications of immunoglobulin G (IgG) in form of monoclonal antibodies and pooled immunoglobulin G preparations has directed attention to this class of glycoproteins. It is commonly appreciated that oligosaccharides attached to the Fc-region play a critical role in the biological activity of IgGs. Thus, glycosylation has been a focus of interest for many scientists and the biopharmaceutical industry and expression hosts have been engineered in order to optimize antibody products. In this review we focus on efforts towards a targeted manipulation of IgG-Fc N-glycans using non-mammalian expression hosts, i.e. yeast, insect cells and plants. Current achievements in generating human-like N-glycan structures will be presented and recent data on the molecular mechanisms that might explain how these potent drugs mediate *in vivo* activities will be discussed.

Introduction

Glycosylation, the attachment of sugar moieties to a given protein backbone, is a largely conserved posttranslational modification in multicellular organisms. In humans, more than 50% of proteins are estimated to be glycosylated [1]. Especially serum glycoproteins form a heterogeneously glycosylated mixture of an otherwise homogeneous protein backbone (microheterogeneity). The glycosylation profile of these proteins is determined by the amino acid sequence, by the conformation at the glycosylation site, by the presence of glycan modifying enzymes and by the availability of suitable activated sugar substrates. In contrast to other biosynthetic functions like DNA-, RNA- or protein synthesis, glycosylation is not under direct transcriptional control and not based on a template. Given the high number of possible glycans attached to proteins, manifold functions can be attributed to the carbohydrate moiety: folding, stability, conformation, solubility, quality control, half-life, oligomerization or functionality. Thus, (proper) glycosylation is vital for most eukaryotes and proteins with specific N-glycosylation patterns are needed in research as well as for medical applications.

Immunoglobulins (Igs)[1] are prominent examples for serum glycoproteins. Depending on the immunological response 5 different Ig-isotypes exist in humans with unique structural and functional properties (further details see [2, this issue]). Some of the isoforms carry up to 7 glycosylation sites (e.g. IgE) and oligosaccharide structures can account for 10–20% of the molecular weight [3, personal communication Friedrich Altmann, BOKU Wien, Austria]. Sequence alignment between different immunoglobulin classes and subclasses indicates the presence of a homologous N-glycosylation site in all of them, except IgA [4]. This conservation indicates an important role of the N-glycan attached at this specific site for structural integrity and/or function of Ig-Fc domains [2, this issue, 5].

Immunoglobulins show a considerable microheterogeneity regarding their glycans. Taking the large human glycome into account, this microheterogeneity may comprise several hundred glycoforms and is mainly owed to the presence or absence of sialic acid, galactose, core-fucose and bisecting N-acetylglucosamine (GlcNAc) [5–7]. IgG, the simplest immunoglobulin isoform, contains one single N-glycosylation site in the constant domains (Asn^{297}), representing the conserved site present in most Ig-classes. For IgGs, Jefferis [2, this issue, 6] estimated a theoretical number of 128 neutral IgG-glycoforms not including charged residues like sialic acid. The oligosaccharide composition of IgGs, the predominant antibody class present in serum, is relatively well characterized [e.g. [8,9]]. Studies of the Fc-N-glycans of serum IgG from healthy individuals revealed several unique characteristics, like a very low degree of sialylation [recently reviewed by Kobata [10]]. This comes as a surprise, since most other serum glycoproteins are highly sialylated. However, as discussed by Jefferis [2] (this issue) the glycosylation pattern of serum IgG can vary dramatically. Differences in IgG glycosylation were noticed e.g. during different diseases, pregnancy and ageing, indicating that some of these variably present glycan residues might play a role in fine-tuning the antibody activity and thus contribute to an optimal immune answer [11]. This microheterogeneity clearly complicates the investigation of the specific functionalities conferred by a

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[1] *Abbreviations used:* Igs, immunoglobulins; IgG, immunoglobulin G; mAbs, monoclonal antibodies; ER, endoplasmic reticulum; FcγR, Fcγ receptor; XT, β1,2-xylosyltransferase; ADCVI, antibody dependent cell-mediated virus inhibition assays.

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single N-glycan residue. The purification of one glycoform from a mixture of only a few different ones might already be challenging [12], not even taking into account the high microheterogeneity of human serum immunoglobulins. Still, the availability of proteins carrying one single oligosaccharide structure can be of high importance for therapeutics, where different glycoforms show different functionality, as in the case of IgGs. There, the absence or presence of core fucose within the Fc-glycan has been linked to the affinity for the Fc receptor and thus the strength of effector functions [13,14]. The reason for this impact has recently been shown to lie in the interaction between the N-glycans of IgG and receptor [15]. This interaction can only take place in an optimal way when the IgG is devoid of core fucose.

Apart from producing more effective drugs, researchers are also dependent on pure glycoforms in their efforts to link specific functions to specific glycosylation patterns. Thus, if purification from a heterogeneously glycosylated mix is impossible, production of single glycoforms is of utmost importance. Currently, most therapeutic monoclonal antibodies (mAbs) are produced in mammalian cell lines (CHO, NS0, SP2/0, ...). In contrast to the 30–40 glycoforms normally detected in human IgG, mammalian cell-derived mAbs carry usually only 5–9 different N-glycan structures and some human-like oligosaccharides are insufficiently produced, if at all (e.g. bisected, di-galactosylated or sialylated structures) [9]. Moreover, it is currently virtually impossible to obtain single glycoform-mAbs from mammalian cell lines. These observations reveal shortcomings of mammalian expression platforms, limited availability of naturally present serum glycoforms and too high glycan-heterogeneity. Success to overcome these limitations is relatively modest (intensively discussed by Jefferis [2, this issue, 5]) as the large, endogenously present glycome complicates a targeted manipulation of the glycosylation profile of mammalian cells.

As outlined above and by Jefferis [2, this issue], the availability of single glycoforms is needed in fundamental research and medicine. This review focuses on strategies for the *in vivo* synthesis of custom-made IgG glycoforms with low glycan-heterogeneity in non-mammalian expression hosts and highlights recent findings in this area. A special focus is given to plant glycoengineering.

Glycosylation pathways

Protein biosynthesis is a relatively conserved process, thus allowing the expression of heterologous proteins in a wide variety of hosts like bacteria, yeast, insect and mammalian cells and plants. N-glycosylation on the other hand can vary strongly between species, between different cell lines of the same species or even between different culture conditions. Notwithstanding these obstacles, the demand for advanced glycoprotein expression platforms has fueled different glycoengineering approaches.

In common with mammalian cells, N-glycosylation of newly synthesized proteins in insect, yeast and plant cells is initiated in the endoplasmic reticulum (ER) by the oligosaccharyltransferase complex, which transfers the oligosaccharide precursor $Glc_3Man_9GlcNAc_2$ onto the growing polypeptide chain, more specifically, onto a suitable asparagine residue within the N-glycosylation consensus sequence (Asn-Xaa-Ser/Thr). This precursor is subsequently trimmed to $Man_8GlcNAc_2$ (Man8) and the protein is then transferred to the Golgi, where further processing occurs (Fig. 1; oligosaccharide abbreviations see: www.proglycan.com). At the point of Man8 the N-glycosylation pathway between yeasts and other eukaryotes diverges. In yeasts, further mannose residues are attached resulting in high-mannosidic structures while in other eukaryotes Man8 first undergoes further trimming and can then be extended with GlcNAc, galactose, sialic acid, fucose and/or xylose to give rise to hybrid, complex or paucimannosidic N-glycans (Fig. 1). Despite these species-specific differences in N-glycosylation, engineering of the respective pathways in non-mammalian expression hosts delivered remarkable results.

Fc glycoengineering in yeast

As an intensively used production platform for recombinant proteins and because of the ease to manipulate the genome by homologous recombination, yeast (particularly *Saccharomyces cervisiae*, *Pichia pastoris*) was one of the first and most successful targets of glycoengineering. Like other eukaryotes, yeasts synthesize ER located Man8 oligosaccharides. Upon translocation to the Golgi, several mannosyltransferases elongate Man8, resulting in large hypermannsoylated glycans that can contain over 100 mannose residues. Such glycoforms are not present in humans, they are thus potentially immunogenic, might alter protein functions and lead to a reduced half-life of therapeutic products. In order to direct the yeast glycosylation pathway towards the synthesis of human-like structures two major issues had to be envisaged: (i) elimination of yeast-specific glycosylation reactions and (ii) introduction of the missing compounds of the human pathway. Indeed, knock out of yeast mannosyltransferase genes prevented hypermannosylation and provided appropriate acceptor substrates for further processing steps [16]. Finally, overexpression of a series of glycan modifying mammalian enzymes allowed the reconstruction of the human glycosylation pathway, including terminal sialylation [17]. To date, this work stands as one of the most remarkable achievements in glycan engineering.

In the course of the humanization of the yeast N-glycosylation pathway seven different, to a large extent pure glycoforms of the cancer-specific mAb rituximab were produced and functional activities were compared to commercial Rituxan [14]. Two of the structures were of complex type, without and with galactose (GnGn and AA, respectively), two carried oligo-mannosidic structures, which represent human intermediates (Man8, Man5). Further forms comprised the tri-mannose core structure Man3 (as typical for insect cells, see below), a hybrid form (Man_5Gn) and wild-type yeast hypermannosylated mAb. Notably, all yeast oligosaccharides lacked core fucosylation while about 90% of Rituxan N-glycans contained this glycan moiety. Fcγ receptor (FcγR) binding studies using different Fcγ receptors revealed that different rituximab glycoforms show a clearly different binding affinity for FcγRs. This variation was particularly pronounced for the low affinity receptor variant FcγRIIIa-F158, where binding affinity could be raised over 100 fold for GnGn and AA carrying mAbs as compared to commercial Rituxan. In addition, improved activities of glycoengineered rituximab that carried GnGn and Man_5Gn structures were confirmed in B-cell depletion assays, which measure antibody-dependent cell killing [14]. This was the first report demonstrating the *in vivo* generation of different mAb glycoforms at great uniformity. The study clearly demonstrates the altered performance of different glycoforms, verifying observations made with mammalian cell-derived mAbs [18,19]. The possible applicability to industry was shown by a recent study demonstrating commercially viable production levels for mAbs produced in glycoengineered yeast [20]. However, pharmacokinetic and clinical studies of such glycoengineered mAbs are pending.

Fc glycoengineering in insect cells

The baculovirus-insect cell expression system is a versatile and efficient eukaryotic expression system particularly well suited for posttranslationally modified proteins [21]. Different glycosylation reactions than in mammalian cells can, however, pose a problem for the therapeutic use of mammalian glycoproteins produced in

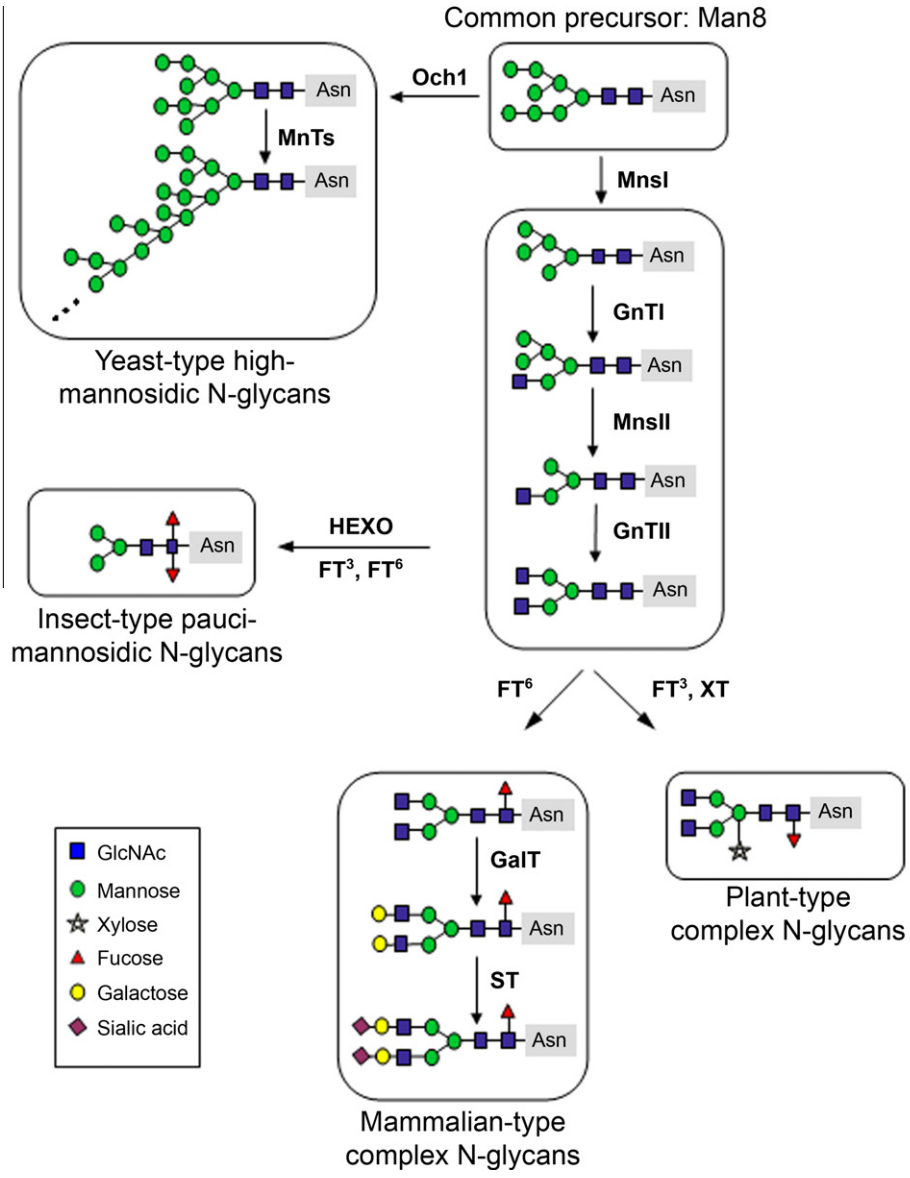

Fig. 1. Schematic presentation of selected components of the N-glycosylation pathways in humans, yeast, insect cells and plants. The common ER-resident oligosaccharide precursor Man8 acts as starting point for further modifications along the Golgi apparatus. Och1: α1,6-mannosyltransferase; MnTs: mannosyltransferases; Mns: mannosidase; GnT: N-acetylglucosaminyltransferase; GalT: α1,4-galactosyltransferase; ST: α2,6-sialyltransferase; HEXO: hexosaminidase (N-acetylglucosaminidase); XT: β 1,2-xylosyltransferase; FT: core fucosyltransferase; Fucose can be transferred in α1,3-linkage (plant typical) and α1,6-linkage (mammalian typical). Interestingly, both forms are synthesized in insect cells. Oligosaccharide abbreviations according to www.proglycan.com.

insect cells. While the processes taking place in ER and early Golgi are very similar, further steps differ [22,23]. Formation of complex N-glycans is initiated – as in mammalian cells – by the addition of an N-acetylglucosaminylresidue to the intermediate Man5 structure and subsequent cleavage of mannose residues. However, in contrast to mammalian cells, further processing comprises trimming of GlcNAc residues by hexosamminidases (HEXO) resulting in the generation of insect-typical paucimannosidic N-glycans. In addition, fucose can be attached to the innermost GlcNAc residue in α1,6- and in α1,3-position (Fig. 1), the latter being absent in mammals. The non-mammalian structures present on insect-cell derived glycoproteins may reduce the *in vivo* bioactivity of therapeutics and might even lead to immunogenic or allergenic reactions [21]. Thus, substantial work has been done to modify N-glycan processing pathways in insect cells in order to generate mammalian-like N-glycans. This finally led to the production of glycoproteins with mammalian-type glycosylation, including protein sialylation [24,25]. However, only a few reports investigate the potential biological impact of insect-specific glycosylation. For IgGs, insect-type Fc-glycosylation does neither significantly alter antigen-binding nor effector functions like CDC when compared to CHO-produced counterparts [26]. On the other hand, mAbs with paucimannosidic N-glycans have been shown to mediate ADCC at significantly lower (50- to 100-fold lower) effector-to-target cell ratios [27]. Further investigations are needed to fully judge the performance of insect cell-derived (glycoengineered) mAbs. Recent results demonstrating the efficient generation of different mAb glycoforms in newly developed insect cells will allow to fill that gap in the near future [28].

Fc glycoengineering in plants

Plants have a longstanding history for the expression of therapeutically relevant recombinant proteins [29]. Due to the largely

conserved secretory pathway between plants and mammals even highly complex molecules, like IgGs and IgAs, can be efficiently produced [30]. Drastic increases in production speed and yield of recombinant proteins have placed plants in a favourable position [31,32]. Particularly the fast production of grams of purified mAbs within a few days after delivery of the appropriate DNA construct to plants [33] provides unique advantages in cases where production speed is of utmost importance, as recently reported for the generation of individualized idiotype IgGs [34].

In contrast to other expression platforms, plants provide the advantage that they synthesize mammalian-type complex N-glycans. *En block* transfer of the $Glc_3Man_9GlcNAc_2$ precursor onto the growing protein and subsequent trimming in ER and *cis/medial*-Golgi compartments are virtually identical in mammals and plants up to the synthesis of GnGn structures (Fig. 1). Beyond that point the glycosylation pathways diverge. In mammals, GnGn structures undergo intensive elongation/modification processes, while plants normally do not further elongate the outer arms of the glycans. On the other hand, plant cells add xylose in β1,2-position to the innermost mannose residue and fucose in α1,3-position to the innermost GlcNAc residue of the GnGn core oligosaccharide (Fig. 1). These residues are not present in mammalian cells, and are consequently, due to reasons discussed above, undesirable. Due to the largely conserved N-glycosylation pathway between mammals and plants the latter provide good prerequisites for glycoengineering. Importantly, mAbs produced in plants exhibit a N-glycosylation profile with a single dominant oligosaccharide structure, $GnGnXF^3$. Pioneering work towards the humanization of the plant glycosylation pathway was carried out by Palacpac et al. [35] and Bakker et al. [36,37]. The two groups overexpressed the human β1,4-glacatosyltransferase (GalT) in tobacco plants and cells in order to elongate the plant-typical $GnGnXF^3$ by β1,4-galactose. This approach resulted in galactosylated structures and it drastically reduced the degree of xylosylation and fucosylation. Although these proof of concept studies demonstrated the ability of plants to synthesize human-type structures by simply overexpressing a mammalian glycosylation enzyme, the overall mAb glycosylation pattern was far from optimal, exhibiting unexpected glycoforms, like incompletely processed and hybrid structures [35–38].

Another major breakthrough towards the humanization of the plant N-glycosylation pathway was the generation of mutants lacking plant-specific β1,2-xylose and core α1,3-fucose. This was achieved by the elimination of the responsible endogenous enzymes, β1,2-xylosyltransferase (XT) and core α1,3-fucosyltransferase (FT^3, Fig. 2). Using knock-down and knock-out approaches for the respective genes, mutant plant lines of *Arabidopsis thaliana*, *Lemna minor*, *Nicotiana benthamiana* and the moss *Physcomitrella patens* were generated (ΔXT/FT plants) and used for the production of different mAbs [39–44]. Notably, the Fc-N-glycosylation profiles of these mAbs contained GnGn as a single dominant structure, with no detectable β1,2-xylose or α1,3-fucose residues remaining. Biological activity assays of these glycoengineered mAbs revealed unaffected antigen binding and CDC activity, however, significantly enhanced ADCC potency as compared to mAbs produced in wild-tpye plants and CHO cells was observed [39,42,45]. These results are in accordance with those obtained using fucose free mAbs produced in other expression platforms. Surprisingly, despite the availability of different expression platforms that permit the generation of such glycoengineered mAbs (yeast, mammalian cells, plants) a direct comparison of their functional activities has not been reported. Thus, at the moment, their performance cannot be fully judged yet.

Elimination of β1,2-xylose and core α1,3-fucose not only demonstrated the ability of plants to synthesize human-type structures without showing any obvious phenotype but also permitted the generation of GnGn, the substrate for further modifications in mammals, such as galactosylation, sialylation, branching, introduction of a bisecting GlcNAc or fucosylation (Figs. 1 and 2).

Targeting of the GalT to a late Golgi compartment significantly improved β1,4-galactosylation in ΔXT/FT plants. mAbs produced in such glycoengineered plants exhibited a single dominant Fc-N-glycan structure, namely digalactosylated AA [45,46], an oligosaccharide generated in insufficient amounts by most mammalian production lines, however dominant in serum IgG [9]. Interestingly, mAbs against HIV produced in such glycoengineered plants exhibited improved anti-viral activity as determined by cell-based virus neutralization assays [45]. Several conflicting reports have been published dealing with the role of terminal galactose residues in modulating IgG activity [11]. As discussed by Jefferis [2, this issue] the degree of antibody galactosylation in the serum can vary during pregnancy and aging and is in certain cases associated with diseases. This observation indicates an active role of this N-glycan residue in modulating IgG activity *in vivo*. Glycoengineered plants as described above provide a suitable platform for the production of digalactosylated antibodies to further investigate the importance of this abundant IgG glycoform.

A minor, yet frequently found glycan moiety on human serum IgG is bisecting GlcNAc, i.e. GlcNAc bound in β1,4-position to the innermost mannose residue [9]. To date no clear contribution to mAb or IgG activities have been assigned to this oligosaccharide residue. One publication [47] reported enhanced ADCC activity of mAb CAMPATH-1H when containing increased amounts of bisected structures. For glycoengineered Rituxan and Herceptin containing an elevated portion of bisecting GlcNAc an enhanced ADCC activity has been reported as compared to mAbs produced in non-modified cells [48]. However, these results are in conflict with

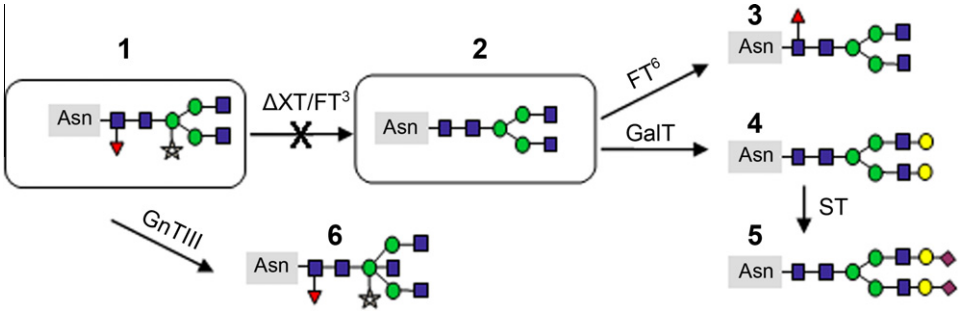

Fig. 2. Fc-Glycoengineering in plants. Overview of mAb glycoforms generated in glycoengineered *Nicotiana benthamiana*, a tobacco related plant species widely used for recombinant protein production. IgG N-glycans generated (1) in wild-type plants: $GnGnXF^3$ [59]; (2) in $\Delta XT/FT^3$ plants: GnGn [59]; (3) in $\Delta XT/FT + FT^6$: $GnGnF^6$ [55]; (4) in ΔXTFT + GalT: AA [45,46]; (5) in ΔXT/FT along with six mammalian genes of the mammalian sialic acid pathway: NaNa [57]; (6) in wild-type + GnTIII: $GnGnXF^3$bi [46]. FT^6: α1,6-fucosyltransferase, GalT: β1,4-galactosyltransferase, ST: α2,6-sialyltransferase, GnTIII: N-acetylglucosaminyltransferase III. Glycan abbreviations according to www.proglycan.com.

others, who do not detect any functional improvements in two different mAbs upon increasing the bisected fraction [49]. The change in ADCC seems rather owed to an decrease in α1,6-fucosylation caused by blocking of the fucosyltransferase through attachment of the bisecting GlcNAc residue [50]. The synthesis of bisected structures in CHO cells – achieved by the overexpression of the corresponding enzyme N-acetylglucosaminyltransferase III (GnTIII) – led to the generation of additional, atypical hybrid structures with significantly reduced core-fucose content [18,51]. Thus, investigating the effect of bisected residues on the function of mAbs remains difficult using exclusively mammalian cells.

Initially, overexpression of GnTIII in plants resulted in a similar observation as in mammalian cells, mAbs carried bisected oligosaccharides, however accompanied by atypical, incompletely processed structures [52]. Interestingly, such hybrid structures can be avoided by targeting the enzyme to a later stage of the plant glycosylation pathway [46]. Using targeting sequences that direct the enzyme to the *trans*-Golgi compartment resulted in the generation of mAbs carrying around 40% fully processed, bisected structures. As addition of bisecting GlcNAc inhibits further oligosaccharide processing in mammalian cells [50], elongation/trimming of N-glycans obviously needs to be completed prior to GnTIII activity in order to obtain complex, bisected oligosaccharides. Surprisingly, in the ΔXT/FT mutant, which lacks plant-specific core modifications, smaller amounts of bisecting glycoforms were synthesized as compared to wild-type plants [46]. An observation, which cannot be entirely explained. Nevertheless, the generation of mAbs carrying large fractions of bisected $GnGnXF^3$bi structures now paves the way to further investigate the contribution of this oligosaccharide moiety to the modulation of mAb or IgG activities.

In recent years manipulation of core α1,6-fucosylation came into the focus of interest, since major contributions to mAb activities have been assigned to that N-glycan residue (detailed description see [2, this issue]). Several studies have been published attempting the modulation of this N-glycan residue in mammalian cells. However, to date it is not possible to generate mAbs in mammalian cells with identical N-glycosylation profiles differing only in the presence/absence of core fucose. Usually the removal of fucose is accompanied by additional changes in the overall N-glycosylation pattern [18,53,54]. Thus, it cannot be excluded that these differences may contribute at least to some extend to altered mAb activities. However, using glycomodified ΔXT/FT plants it was possible to completely restore mammalian-type core α1,6-fucosylation by overexpressing the responsible mammalian enzyme, core α1,6-fucosyltransferase (Fig. 2). This allowed the generation of mAbs with and without fucose while maintaining an otherwise identical N-glycosylation pattern [46,55]. In the course of these experiments the impact of Fc-glycosylation on the antiviral activity of the broadly neutralizing HIV-1 mAb 2G12 was investigated. Different plant-derived 2G12 batches exhibited glycosylation profiles containing a single dominant N-glycan structure, amongst them 2G12 carrying $GnGnXF^3$, $GnGnF^6$, GnGn and digalactosylated AA structures. CHO-derived 2G12 on the other hand carried a mixture of six N-glycans. The plant-derived 2G12 glycoforms differed only in one or two glycan residues, thus allowing precise investigations of the impact of single glycan moieties on Ab activity [55]. All 2G12 glycoforms exhibited similar binding to FcγRI, FcγRIIa, and FcγRIIb. In contrast, binding of 2G12 to FcγRIIIa was markedly affected by core fucose, irrespective of its plant-specific α1,3- or mammalian-type α1,6-linkage. Consistent with this finding, 2G12 glycoforms lacking core fucose mediated higher antiviral activity against various lentiviruses (incl. HIV-1) as measured by antibody dependent cell-mediated virus inhibition assays (ADCVI), an equivalent to ADCC induction by anti-cancer mAbs. In addition the results indicate that further elongation of GnGn by terminal β1,4-galactose (AA structures) does not significantly enhance 2G12 ADCVI. So far this is the only study that investigated *in vitro* and *in vivo* antiviral activity of glycoengineered mAbs, pointing to the importance of optimal N-glycosylation for immunotherapeutic reagents [55].

The final and most complex step of human N-glycosylation is terminal sialylation. There is long-standing evidence that IgG molecules can have an anti-inflammatory activity in autoimmune diseases and recent studies indicate that this activity is associated with the presence of sialic acid (for further description see [2, this issue, 56]). However, detailed studies that back this important phenomenon are hampered by the difficulties in obtaining highly sialylated IgG, a glycoform present only in low quantities in serum and inefficiently synthesized in mammalian cells. Moreover, CHO cells naturally attach sialic acid in α2,3-position, whereas α2,6-linkage is the preferred type on serum IgG.

Sialylation is particularly difficult to accomplish in plants because plants lack some essential prerequisites along the

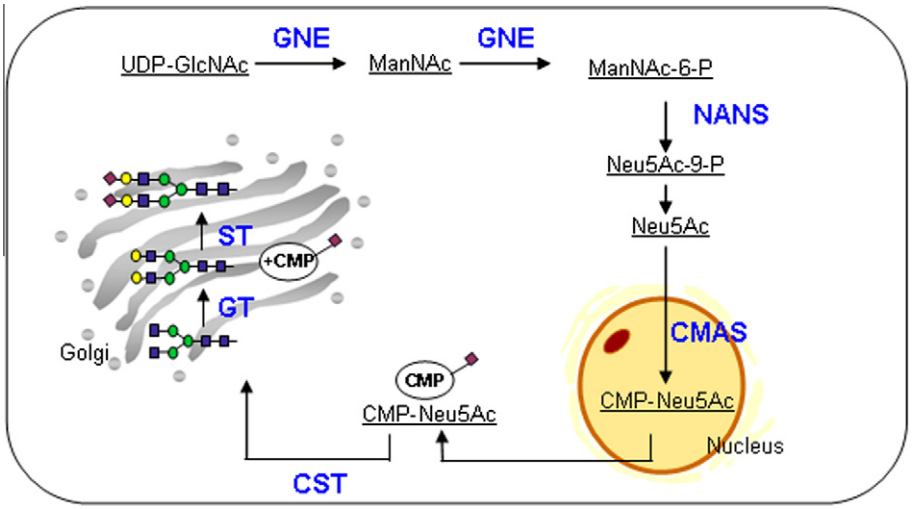

Fig. 3. Reconstruction of the human sialylation pathway in plants using the endogenously present metabolite UDP-GlcNAc. Enzymes involved in the process are: UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine-kinase (GNE), N-acetylneuraminic acid phosphate-synthase (NANS), CMP-sialic acid (Neu5Ac) synthetase (CMAS), CMP-Neu5Ac transporter (CST), β1,4-galactosyltransfease (GT) and α2,6-sialyltransferase (ST). *In planta* protein sialylation was achieved by the coordinated expression and correct subcellular deposition of genes/proteins for (i) biosynthesis (GNE, NANS), (ii) activation (CMAS), (iii) transport (CST), and (iv) transfer of Neu5Ac to terminal galactose (ST) [57]. Glycan abbreviations according to www.proglycan.com.

biosynthetic pathway (Fig. 3). Despite these shortcomings, *in planta* sialylation of mAbs was recently reported [57]. Castilho et al. had introduced enzymes of the mammalian pathway into plants, allowing the biosynthesis of sialic acid, its activation, its transport into the Golgi, and finally its transfer onto terminal galactose (Fig. 3). mAbs coexpressed with the genes from the human sialylation pathway carried up to 80% sialylated structures [57]. A remarkable number considering the required coordinated overexpression of six mammalian enzymes which act at various stages and in different subcellular compartments of the biosynthetic pathway. Notably, *in planta* sialylation did not have a negative impact on mAb expression, which is remarkable in the light of the complexity of both processes.

At the time this review is written experiments are underway that investigate possible contributions of Fc sialylation to IgG activities.

Conclusion

To fully understand the different immunoglobulin activities, more than protein–protein interactions alone have to be considered. The oligosaccharide moiety of IgG-Fcs has shown to wield influence on their various functions, indicating that this glycan is more than a simple building block required solely for correct three dimensional conformation and stability. The absence or presence of this glycan or even of single sugar residues can strongly influence the mode of action and the different glycoforms present in serum during different physiological stages, e.g. disease, aging or pregnancy, indicate a certain level of active control over the N-glycosylation machinery. Only now we are starting to uncover the factors that govern differential N-glycosylation and how N-glycans affect different IgG functions. In this respect, access to pure glycoforms is a crucial prerequisite enabling more detailed structure–function studies that will have an impact on medicine as well. For example, IgGs lacking core fucose have been shown to possess a drastically improved *in vivo* activity and thus have raised interest as next-generation mAbs with improved efficacy [54,58]. The effects of other naturally occurring Fc-glycoforms however remain largely unknown. The generation of different single glycoforms will further increase our knowledge of structure–function relations of IgG glycosylation and allow the production of mAbs with even higher clinical potency. The here described glycoengineered expression platforms will significantly contribute to advances in this field.

Acknowledgments

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