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Review

Alternative glycosylation modulates function of IgG and other proteins — Implications on evolution and disease ☆

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ABSTRACT

Background: Nearly all membrane and secreted proteins, as well as numerous intracellular proteins are glycosylated. However, contrary to proteins which are defined by their individual genetic templates, glycans are encoded in a complex dynamic network of hundreds of genes which participate in the complex biosynthetic pathway of protein glycosylation.
Scope of review: This review summarizes present knowledge about the importance of alternative glycosylation of IgG and other proteins.
Major conclusions: Numerous proteins depend on correct glycosylation for proper function. Very good example for this is the alternative glycosylation of IgG whose effector functions can be completely changed by the addition or removal of a single monosaccharide residue from its glycans.
General significance: The change in the structure of a protein requires mutations in DNA and subsequent selection in the next generation, while even slight alterations in activity or intracellular localization of one or more biosynthetic enzymes are sufficient for the creation of novel glycan structures, which can then perform new functions. Glycome composition varies significantly between individuals, which makes them slightly or even significantly different in their ability to execute specific molecular pathways with numerous implications for development and progression of various diseases. This article is part of a Special Issue entitled Glycoproteomics.

1. Introduction

Sequencing of the entire human genome was a great achievement of the human mind, but the fact that the number of our genes exceeds the number of genes in a simple bacteria by only a factor of five, or that we have practically the same number of genes as a mouse or a zebrafish, was surprising and entirely unexpected [1–3]. Humans are clearly more than five times more complex than bacteria, but proteins are apparently not the place where one should look for the source of human complexity. This revelation opened a new way of looking at proteins and genes which code for them. Instead of being decisive in shaping all processes which occur in the body, genes and their protein products appear to be more like tools which perform dedicated, simple, or more complex tasks [4]. If proteins are considered to be analogous to basic tools like wrenches, hammers and saws, or more complex tools like cars, trucks or canons, then 25,000 different tools are probably more than plenty to perform all tasks for a complex eukaryotic organism. As the set of very similar tools can be used to make a small motorbike or a fancy racing car, the set of very similar protein coding genes can be used to make both mouse and man. It is not the tools which decide what will the final product be, but the time, place and way they are being used. This is the point where all complex new biology like glycosylation and other posttranslational modifications, epigenetics and numerous types of non-coding DNA, inheritance of acquired characteristics and numerous additional still unknown mechanisms come into place and generate the full complexity of life which exists today.

2. Protein glycosylation is a very complex process

The assumption that function of each protein is determined by its structure, which is defined by the sequence of nucleotides in the corresponding gene, is the central dogma of biology which still stands. However, in the case of glycan parts of glycoproteins this dogma has to accommodate several additional levels of complexity which lay between genes and glycans. Genes determine the structure of proteins which participate in the process of glycan synthesis, but in addition to genetic polymorphisms in the participating genes, regulation of gene expression, posttranslational modifications, and the activity of the corresponding proteins work together to shape the final structure of a glycan. Additional mechanisms including altered intracellular localization, competition with endogenous acceptor substrates and variable access to monosaccharide donor substrates can also affect the final structure of a glycan [5].

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Nearly all membrane and secreted proteins [6], as well as numerous cytoplasmic proteins [7,8] are glycosylated. However, glycosylation is not a simple addition of glycan decorations, but an intricate carefully regulated process which results in the creation of specific branched oligosaccharide chains (glycans) which significantly affect protein structure and function. Hundreds of glycosyltransferases are involved in protein glycosylation [9], but they are probably only a tip of the iceberg of protein glycosylation. Recent study of neural-specific glycosylation in drosophila revealed that less than 13% of genes required for successful glycosylation were previously known glyco-genes [10]. Direct extrapolation of this finding is not possible because many of these other proteins will also participate in other types of glycosylation, but it is very probable that at least two or three times more genes are needed for protein glycosylation than the currently listed 600 glyco-genes [9]. Therefore, at least between 5% and 10% of the human genome is needed to glycosylate proteins what makes protein glycosylation the most complex biosynthetic pathway. Recently initiated genome wide association studies (GWAS) of the glycome [11,12] are expected to map the regulatory network of protein glycosylation in the near future [13].

At the population level there is a large variability in the composition of the individual's glycomes [14] which originates from both inherited genetic polymorphisms and different past and present environmental effects, which act either directly, or through acquired epigenetic regulation [15,16]. The majority of human variability originates from single nucleotide polymorphisms (SNPs) that individually do not have visible phenotypes, but if present in specific combinations within the same individual, can have significant phenotypic effects [17–20]. Due to hundreds of genes that interact to generate glycans, glycosylation is particularly prone to this type of cumulated variability. Some combinations of individual SNPs can be manifested as specific glyco-phenotypes, which might represent potential evolutionary advantages or disadvantages [21]. The most prominent example is the various forms of congenital disorders of glycosylation [22] which are usually caused by a combination of several individual mutations [23]. Most of these mutations are leaky mutations with some residual enzyme activity. However, when combined in the same individual they result in a complex phenotype associated with significant mortality and extensive motoric, immunological, digestive and neurological symptoms [22,24].

N-glycosylation is essential for multicellular life and its complete absence is embryonically lethal [25]. However, mutations in genes involved in modifications of glycan antennas are common and apparently cause a large part of individual phenotypic variations that exist in humans and other higher organisms. The most prominent example is the ABO system of blood group determination, which arises from the existence of three allelic variants of a single glycosyltransferase gene. The variability of plasma glycome composition between individuals is very large [14], indicating that genetic variability and past events significantly affect glycome composition. Changes in the composition of the plasma proteome cannot be excluded as a source of glycome variability when the total plasma glycome is being analyzed, but studies of the isolated IgG revealed even larger variability [26], indicating that even glycosylation of a single protein varies significantly between individuals. However, this variability is clearly within some physiological limits because it was demonstrated that cells have highly organized glycomic compensation systems which preserve N-glycan branch complexity even when multiple genes are silenced in parallel [27].

3. Alternative glycosylation modulates function of IgG

Alternative glycosylation is a term which is seldom used to describe posttranslational modifications of a protein. Until recently it was widely believed that different glycans which can be attached to the same glycosylation site of a protein are simply a consequence of the absence of the genetic template and the consequential lack of stringency within the biosynthetic pathway. However, as more information about protein glycosylation is becoming available, it is becoming clear that, at least for some glycoproteins, this is clearly not the case. Glycosylation of many proteins, and particularly of IgG, is very strictly regulated and the attachment of different glycans is of great physiological significance. When treated with specific environmental factors or cytokines, B-cells modulate IgG glycosylation in a specific manner [28]. In the same way as alternative splicing of mRNA produces different proteins which have different functions, alternative glycosylation produces different glycoproteins which can have very different functions.

IgG is one of the most studied glycoproteins. This complex protein is an essential tool in the defense against various pathogens, toxins and cancer. While Fab parts of IgG are responsible for recognition of non-self structures, Fc part executes further actions for the removal or destruction of foreign objects through interaction with different Fc receptors [29]. Depending on the nature of the foreign structure, antibodies need to activate different effector pathways and here alternative glycosylation apparently plays an important role by enabling a simple mechanism of structural alterations in the Fc part and consequential preference for different Fc receptors. Recent structural studies using NMR indicated that IgG Fc glycans are more accessible and dynamic than previously thought and that they might play more active role in interactions with Fc receptors [30]. This is further supported by observations that interactions between glycans on Fc receptors and glycans on IgG are a molecular mechanism of the preference of FcγRIII for binding of non-fucosylated IgG [31].

Each heavy chain of IgG carries a single covalently attached bi-antennary *N*-glycan at the highly conserved asparagine 297 residue in each of the C_H2 domains of the Fc region of the molecule. The attached glycans are essential structural components of the Fc region and fine changes in glycan composition can significantly change conformation of the Fc region with dramatic consequences for effector functions of IgG (Fig. 1). For example, the addition of fucose residue to the first N-acetylglucosamine in the core of the glycan (core-fucose) modifies the conformation of the Fc region in a way to dramatically reduce its ability to bind to FcγRIIIa [32–34]. FcγRIIIa (CD16) is an activating Fc receptor expressed primarily on natural killer (NK) cells. Through binding to FcγRIIIa antibodies initiate antibody dependent cellular cytotoxicity (ADCC) which results in destruction of target cells. On average over 95% of circulating IgGs are core-fucosylated [26] and therefore contain "safety switch" which prevents them from eliciting potentially destructive ADCC [35]. This is an unusual feature of IgG, since the majority of other plasma proteins do not contain core-fucose [14]. Small fraction of IgG which lack core-fucose are over 100 times more effective in initiating ADCC through binding to FcγRIIIa and this seems to be the primary mode of function of therapeutic anti-cancer monoclonal antibodies [32,36,37]. Interestingly, glycans attached to Asn45 and Asn162 of FcγRIIIa were found to be essential for the regulation of preferential binding of IgG without core-fucose to FcγRIIIa [38].

Another structural alteration of the IgG glycan, the addition of sialic acid to the ends of glycans practically reverts function of IgG and converts it from being pro-inflammatory into an anti-inflammatory agent [39]. Sialylation of IgG was found to be essential for the function of intravenous immunoglobulin (IVIG). IVIG is a therapeutic preparation of highly purified, polyclonal IgG antibodies which is widely used for the treatment of a number of autoimmune diseases, including immune thrombocytopenic purpura, Kawasaki's disease, Guillain–Barré syndrome, and chronic inflammatory demyelinating neuropathy. Anti-inflammatory activity is contained within the effector Fc portion, as Fc fragments were found to be sufficient to suppress inflammation [40]. However, Anthony and colleagues demonstrated that Fc γ receptors (FcγRs) and neonatal Fc receptor (FcRn) are not involved in this pathway and that SIGN-R1, a C-type lectin receptor on mouse splenic macrophages, recognizes sialic acids on IgG and mediates its anti-inflammatory effects [41]. In addition, they also reported that sialylated IgG Fc proteins also bind to DC-SIGN, the human orthologue of SIGN-R1. Fractionation of Fc by SNA-lectin affinity chromatography indicated

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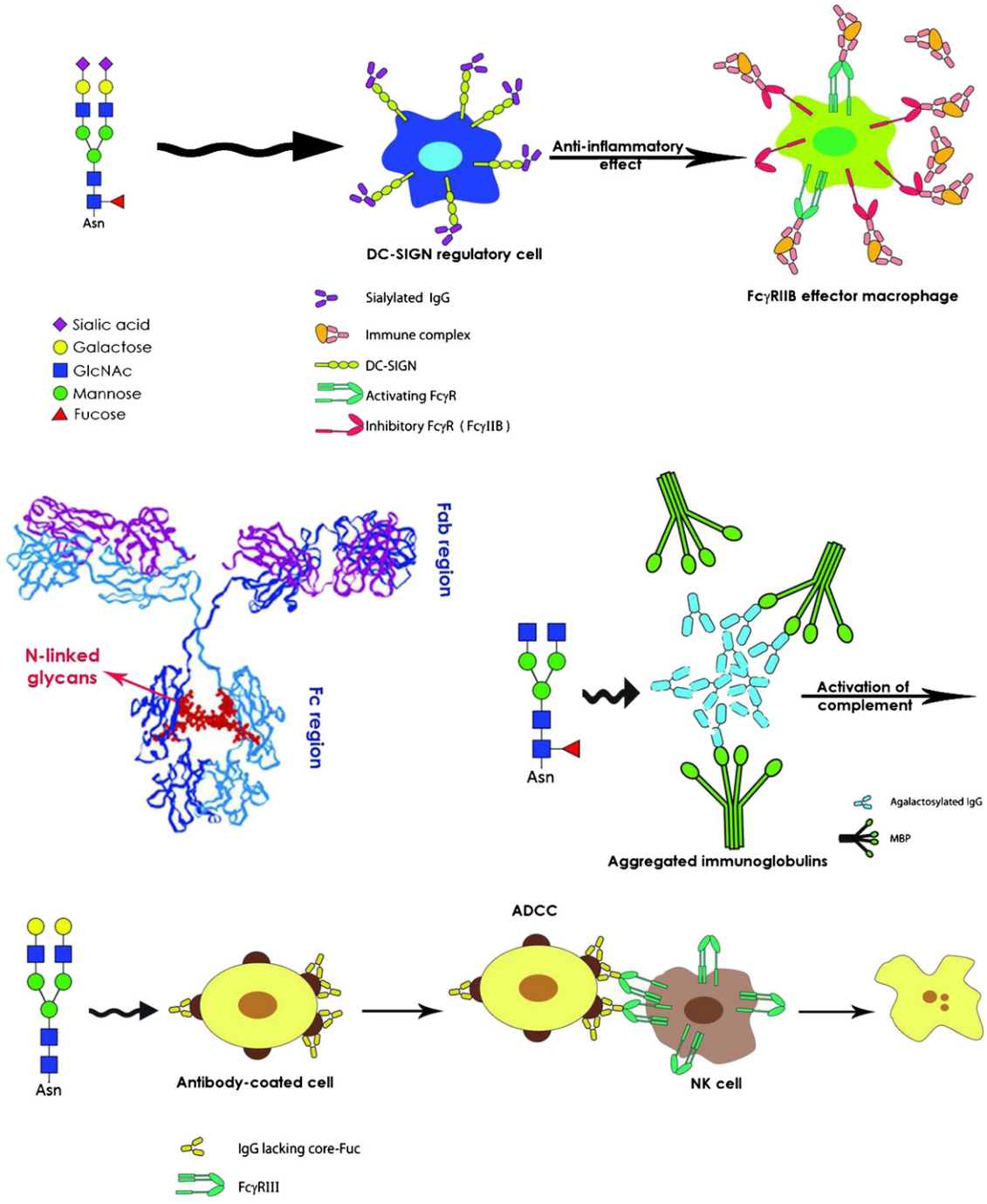

Fig. 1. Modulation of IgG function by alternative glycosylation. Structure of the glycan on IgG Fc part can significantly affect effector functions of IgG. For detailed explanations, see the main text.

that at least two sialic acids are needed for efficient binding [42]. Interestingly, a combined epitope consisting of both IgG peptide and α2,6-sialylated glycan is apparently necessary for binding to DC-SIGN [41], a paradigm which was also demonstrated for binding of P-selectins to glycoprotein ligands [43]. Recent studies indicated that Fc with sialylated glycans suppresses inflammation through a novel T_H2 pathway which provides an intrinsic mechanism for maintaining immune homeostasis that could be manipulated to provide therapeutic benefit in autoimmune diseases [44].

These two examples clearly demonstrate that alternative glycosylation can be used as an effective way of modulating protein structure which enables them to perform completely different functions. The addition of galactose to IgG glycans is also of great biological significance. More than 25 years ago it was reported to be associated with rheumatoid arthritis [45] and since then over 50 different studies have analyzed the role of IgG galactosylation in different inflammatory diseases [46]. Incomplete galactosylation of IgG can activate complement via the mannose-binding protein and thus be part of the underlying pathological mechanism [47,48]. Galactosylation of IgG is undergoing specific changes with aging [26,49] and in a number of different diseases, but the molecular significance of these changes is still mostly unknown [50].

4. Alternative glycosylation of other proteins

Changes in the attached glycans significantly affect the structure and function of polypeptide parts of many glycoproteins [51]. Proper

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glycosylation of membrane receptors is particularly important since it modulates adaptive properties of the cell membrane and affects communication between the cell and its environment [52]. Alternative glycosylation of proteins gives cell an opportunity to quickly react to changes in the environment and adapt properties of its membrane [53]. The association between polyunsaturated fatty acids and highly branched glycans indicating the presence of a compensatory mechanism between these two types of structures was recently observed in a large population study [54]. Understanding of the complex structure/function relationship between polypeptides and attached glycans requires detailed studies of all individual alternatively glycosylated proteins that are technically very difficult. Nevertheless, even with currently available methods a significant pool of evidence demonstrating the importance of glycosylation for proper function of numerous proteins has accumulated (Table 1).

One of the first examples of functionally significant alternative glycosylation was the important developmental regulator Notch which controls a broad range of cell-fate decisions throughout the metazoa [55]. Binding of ligands to Notch receptors in neighboring cells triggers proteolytic processing of Notch, which results in the release of the intracellular domain of Notch from the plasma membrane. In addition to the standard *N*-linked glycosylation, Notch is also modified by two unusual types of O-linked glycosylation; the addition of O-linked glucose (O-glucose) and O-linked fucose (O-fucose) [56]. O-fucose can be extended with β1,3-linked GlcNAc by a glycosyltransferase Fringe what modulates a subset of Notch signaling processes [57,58]. This disaccharide can be further extended, but functional significance of this extension is not fully elucidated [56,59]. However, it is evident that O-fucose glycans of Notch receptors are important modulators of Notch signaling since their removal by mutating relevant glycosyltransferases results in severe, global Notch signaling defects [60].

Another prominent example of the importance of glycosylation for protein function is the glycosylation of glucose transporters (GLUTs). These large transmembrane proteins govern uptake of glucose in the cell using interesting mode of regulation based on shifting fully functional proteins between the cell membrane and intracellular vesicles. In mice, but apparently not in humans [61], the principal glucose transporter in pancreatic beta cells is GLUT-2 and the complete suppression of GLUT2 gene expression suppresses glucose stimulated insulin secretion (GSIS) [62]. Interestingly, the inactivation of the gene for the Golgi N-acetylglucosamine transferase 4a (GnT-4a, product of the *Mgat4a* gene) resulted in nearly identical phenotype [63]. Ohtsubo et al., postulated that the N-glycan structure was required to anchor GLUT-2 at the cell surface through an interaction with a cell-surface lectin. Indeed, in competition experiments, glycans mimicking the structure of the side chain missing in the knockout mice induced GLUT-2 internalization in control islets. Furthermore, the authors identified galectin 9 as the interacting lectin [63]. N-glycosylation is also critical for the stability and intracellular trafficking of GLUT-4 glucose transporter and a specific structural element of *N*-glycan may be critical for the localization of GLUT4 to the appropriate intracellular pool essential for insulin-mediated translocation [64].

The results of lectin-binding analyses of human GLUT-1 and GLUT-2 N-glycans from healthy donors were consistent with the presence of an

Table 1
Examples of proteins which require proper glycosylation for function.

Protein[a]	Effect	Ref.
Acetylcholinesterase (AchE)	N-glycosylation of AChE plays a major role for acquisition of AChE full enzymatic activity, but does not affect its oligomerization (AchE anchors onto membrane by a transmembrane protein PriMA, as a tetrameric form). Site-directed mutagenesis of all N-glycosylation sites results in production of a glycan-depleted PRiMA-linked AChE tetramer, but with much higher Km value compared to the wild-type. The complex is not transported to plasma membrane.	[102]
Cardiac ion channels	Alternative glycosylation affects activity of cardiac ion channels	[103,104]
CD45	O-glycan modifications of CD45 are essential for galectin-1 binding and clustering (positive regulation of T cell death).	[105]
Cystic fibrosis transmembrane conductance regulator (CFTR)	N-glycans, specifically core glycans, enhance the productive folding and conformational stability of CFTR. Defective N-glycosylation reduces cell surface expression by impairing both early secretory and endocytic traffic of CFTR. Conformational destabilization of the glycan-deficient CFTR induces ubiquitination, leading to rapid elimination from the cell surface.	[106]
E-cadherin	N-glycosylation modulates E-cadherin function and dysfunction in cancer. Asn633 glycan is critical for stability, while Asn554 and Asn566 glycans are vital for the tumor-suppressive role of E-cadherin in cell cycle progression.	[107,108]
Epidermal growth factor receptor	Sialylation and fucosylation of epidermal growth factor receptor suppress its dimerization and activation in lung cancer cells	[109]
Fibrinogen	Deglycosylation leads to turbidity development, lateral aggregation, and porosity of clots. Also, deglycosylated fibrinogen is less soluble than normal fibrinogen.	[110]
GABA transporter (GAT1)	N-glycosylation is vital for protein stability and trafficking to the plasma membrane and also for GABA-uptake activity.	[111]
GABAA receptor	Glycosylation of Asn173 is important for stability of β2 subunits when expressed alone and glycosylation of Asn104 is required for efficient α1β2 receptor assembly and/or stability in the ER. Mutation of each site decreases peak α1β2 receptor current amplitudes and alters the gating properties of α1β2 receptor channels by reducing mean open time due to a reduction in the proportion of long open states.	[112]
GLUT1	N-glycosylation contributes to the formation of structure that is competent for high affinity transport.	[113]
Haptoglobin	Carbohydrate portion is essential for the functionally active form of polymeric haptoglobin.	[114]
Human chorionic gonadotropin (hCG)	Asn52 glycosylation site is implicated in signal transduction and heterodimer association, Asn 78 glycan plays a critical role in maintaining the structure of α-subunit.	[115]
Insulin receptor	Complete deglycosylation leads to loss of binding. Alternative glycosylation of the insulin receptor prevents oligomerization and acquisition of insulin-dependent tyrosine kinase activity	[116–118]
Integrin α5β1	Removal of the N-glycosylation sites on the I-like domain of the beta1 subunit decreased both the level of expression and heterodimeric formation, resulting in inhibition of cell spreading; N-glycosylation of the I-like domain is essential to both the heterodimer formation and biological function of the subunit.	[119]
MHC class II molecules	Deglycosylation results in decreased stability, but N-glycan of MHC class II molecules may not be essential for either antigenic peptide binding or T cell recognition.	[120]
Nicotinic acetylcholine receptor	Deglycosylation leads to increased degradation of α subunit, but has no effect on affinities of the 2 α subunits for cholinergic ligands.	[121]
Organic anion transporter (OAT1)	Disruption of Asn39 resulted in an almost complete loss of transport activity without affecting the cell surface expression.	[122]
Plasminogen	Glycosylation is essential for binding to cellular receptors and fibrinolytic activity.	[123]
Transferrin	Sialylation of transferrin is rapidly changing in sepsis and acute pancreatitis	[124–126]
γ-glutamyl transpeptidase (GGT)	N-glycosylation of human GGT is required for the proper folding and subsequent cleavage of the nascent propeptide, although retention of these N-glycans is not necessary for maintaining either the function or structural stability of the mature enzyme.	[127]

[a] More extensive list of proteins which require glycosylation for normal function is available as electronic supplementary material.

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undersialylated tetra-antennary structure bearing the core β1-4GlcNAc glycan linkage produced by GnT-4a [65]. Multiple cell types express GnT-4a with high expression in the pancreas of normal rodents and humans. Nevertheless, beta cell-specific transgene expression markedly diminished signs of high-fat diet-induced diabetes in the presence of endogenous GnT-4a expression. In beta cells, multiple glycoprotein substrates of GnT-4a exist, including the insulin-like growth factor-1 receptor and insulin receptor-α subunit. However, the turnover of these substrates was unaffected by the deficiency of GnT-4a activity and core β1-4GlcNAc glycan linkages [63]. The glucose transporters of beta cells are thus unique (among glycoproteins analyzed) in requiring GnT-4a glycosylation to sustain an extended half-life at the cell surface, suggesting a stabilizing role of lectin–ligand binding that may involve the galectins [66,67]. Both GnT-4a and the glucose transporters are highly regulated in pancreatic beta cells. Their expression is rapidly lost in primary beta cell cultures, and immortalized beta cell lines lose GSIS activity [65]. Epigenetic silencing of HNF1A and the consequential downregulation of GnT-4a were proposed as one of mechanisms leading to diabetes [65], while methylation of *HNF1A* was reported to associate with the increased branching of glycans on human plasma glycoproteins [68]. In general, proper glycosylation of membrane proteins seems to be essential for the normal function of the cell membrane [52,69] and even when multiple glycosyltransferases are silenced in parallel, cells manage to preserve the abundance of terminal glycan moieties, indicating the presence of an intricate glycomic compensation system [27].

5. Evolutionary implications of alternative glycosylation

Complex dynamic network of hundreds of enzymes and other proteins that participate in the synthesis of glycans make the process of protein glycosylation inherently sensitive to all changes occurring within and around the cell. The ability to create novel biological structures without previous changes in the precious genetic heritage is a huge advantage which higher eukaryotes have used extensively in the course of evolution. Changes in glycosylation are also an important evolutionary mechanism for other organisms and even viruses [70], but their relative importance for higher organisms is much higher, because of their much lower reproductive potential. The best proof of universal significance of glycosylation is the fact that surfaces of virtually all living cells are covered by a dense and complex array of cell surface glycans [71]. By combining nine principal monosaccharides into chains of varying lengths and degrees of branching vertebrates produce much more complex branched N-glycans than many other lineages [72,73]. However, unlike linear DNA sequences in which evolutionary events can be more or less successfully tracked and/or detected, this type of analysis is not possible for nonlinear branched glycans. Also, the pace of changes in glycans is much faster than in DNA and closely related organisms can have very different glycans attached to nearly identical polypeptide backbones [74]. Another evidence of the rapid evolution of the glycome was provided by the recent comparison of milk oligosaccharides in various primates which indicated that the composition of milk glycome evolved through the ongoing competition between commensal bacteria and pathogenic bacteria and the need to maintain a protective population of commensal bacteria [75].

A fascinating example of the role of glycans in the interaction between the cell and environment is the modulation of intestinal glycome composition. Commensal bacteria regulate intestinal physiology, development and function [76], and are essential for the maintenance of immune homeostasis in the gut [77]. An active dialog between commensal microflora and the host mucosal glycans apparently affects immunological tolerance and homeostasis within the gut and can explain some of the differential host responses to commensal and pathogenic bacteria [78]. The mechanisms of this phenomenon are not known, but apparently some signals from the cell membrane exposed to specific non-pathogenic commensal bacteria affect the glycosylation machinery and instruct it to produce glycans which promote successful symbiosis with intestinal commensal bacteria [79].

Recently it has been shown that epigenetic changes can influence the expression level of glyco-genes, consequently leading to aberrant glycosylation and expression of cancer-associated carbohydrate antigens [80,81]. Epigenetic regulation is a universal tool that higher organisms use to adapt to changes in the environment [82,83]. While environmental factors like diet influence enzymatic processes only while they are directly present, their prolonged effects can be achieved through the cell memory of epigenetic marks. By modulating expression of relevant genes, epigenetic marks can influence glycan structures and make the organism more fit for a specific environment. The epigenetic regulation of glycosyltransferases in cancer cells results in the creation of novel glycan structures [80,81] that appear to be one of the mechanisms used by cancer cells to evade the host immune response. This clearly demonstrates that novel biological structures can be created without any changes in the DNA sequence of an organism. Though in the case of cancer cells the epigenetic modulation of gene expression is the obvious misuse of glycosylation machinery, it points out to a general evolutionary mechanism available to complex organisms; by altering epigenetic status of different glyco-genes they can produce novel biological structures without the need to change the genetic information itself. Transgenerational inheritance of some epigenetic marks clearly exists, but is still only vaguely understood [82–86]. If acquired epigenetic regulation of genes responsible for glycosylation could also be somehow transmitted to the germ cells, this might be an essential evolutionary mechanism, which would enable adaptation of complex organisms to environmental changes, while preserving the precious genetic heritage.

IgG is a clear example of how novel functions can be developed for an existing protein (gene) by alternative glycosylation. IgG has a particularly difficult physiological task. It has to protect the organism from a number of different current and future known and unknown threats which come from the constantly changing environment. An elaborate genetic system has evolved which enables IgG to efficiently recognize non-self, but after binding to non-self structures IgG has to initiate a response mechanisms which needs to be different for different types of threats [87]. Therefore, after the development of a successful DNA sequence for a variable part of IgG, this variable part (Fab) needs to be associated with an appropriate Fc part of IgG which will bind to the correct Fc receptor and initiate the right kind of immune response. One evolutionary solution for this problem was the development of several different classes of Fc (IgG1, IgG2, IgG3, IgG4) which can than perform different functions. Another, more flexible and dynamic solution for the same problem was the development of alternative glycosylation of IgG Fc as described in the section "Alternative glycosylation modulates function of IgG" of this review.

6. Alternative glycosylation and disease

Complex human diseases are a combination of a genetic background and environmental influence. Genome wide association studies have mapped a number of genetic polymorphisms associated with diseases in the last few years, but even in very large studies comprising hundreds of thousands of individuals, the current approach which is comparing individual SNPs with phenotypes is failing to explain a large part of heritability which is known to exist in complex diseases [88]. New statistical models which will account for various gene–gene and gene–environment interactions will probably be needed to identify more complex relations between genetic polymorphisms and complex diseases [17,89]. Analogously to a complex disease, the composition of an individual's glycome is a combination of genetic background and all relevant past events in the cell [90]. Individual glycan structures vary depending on the current level of expression and intracellular localization of biosynthetic enzymes and altered

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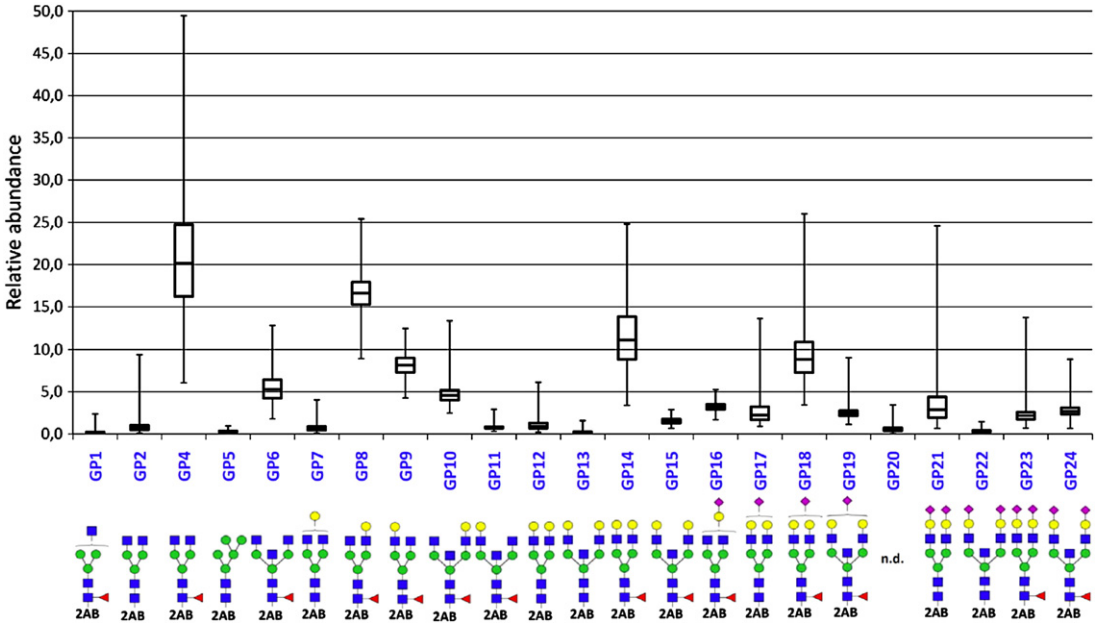

Fig. 2. Variability of IgG glycome composition in a human population. Relative percentages of individual glycans in the total IgG glycome are shown as box-plots. Top and bottom of the box represent 25th and 75th percentile, while lines extend to minimal and maximal values observed in the human population. Structures of glycans in each HPLC peak are shown below the plot (blue square — N-acetylglucosamine; green circle — mannose; yellow circle — galactose; purple diamond — sialic acid; red triangle — fucose). Detailed description of the study is available in the original manuscript [26].

glycan structures are often attached to the same protein backbone as a consequence of different pathophysiological processes. Consequently, various diseases are associated with specific changes in glycan structures. However, the description of numerous glycosylation changes in disease is outside the scope of this manuscript and the interested reader is advised to consult other excellent recent reviews on this topic [46,91–95].

In addition to specific glycan biomarkers which are products of altered cell metabolism in a specific disease, common polymorphisms in the glycosylation machinery and consequential differences in glycome composition which normally exist in the population can also be important diagnostic and prognostic markers. Structural complexity of glycans was a great obstacle for their analysis and until very recently it was virtually impossible to quantitatively analyze glycome composition in a large number of individuals. However, recent development of high throughput methods for analyzing glycans using HPLC [96], mass spectrometry [97,98] and capillary electrophoresis [99] reverted the situation and several studies reporting protein glycosylation in large human populations were published in the last few years [14,21,26,49,100].

The variability in plasma glycome composition [14], and even more in IgG glycome composition [26] was found to be very high. The average ratio of minimal to maximal observed levels of individual glycans in the plasma and IgG glycomes was 6.17 and 17.2, respectively. The large part of this variability originated from less abundant glycans, but even levels of some of the most abundant glycans vary significantly (Fig. 2). To the contrary, the composition of a glycome of any given individual was found to be very stable even after prolonged periods of time [101], indicating strong genetic (including epigenetic) regulation of protein N-glycosylation. Apparently the combination of genetic polymorphisms and environmental effects can result in significantly aberrant glycome composition (glyco-phenotypes) in some individuals and some of these aberrant glyco-phenotypes were reported to be associated with specific diseases [21]. However, reliable association of specific glyco-phenotypes with predisposition for the development or progression of a specific disease requires analysis of thousands of patients. Large scale studies of protein glycosylation in disease were not done yet, but the technology is now available and it is to be expected that specific glyco-phenotypes will be associated with diseases soon.

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Appendix A. Supplementary data

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