

Glycosylation of serum proteins in inflammatory diseases

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Abstract. Inflammatory diseases are accompanied by numerous changes at the site of inflammation as well as many systemic physiological and biochemical changes. In the past two decades more and more attention is being paid to changes in glycosylation and in this review we describe some of the changes found on main serum proteins (α 1-acid glycoprotein, immunoglobulin G, immunoglobulin A, transferrin, haptoglobin, α 2-macroglobulin, C-reactive protein, and others). Molecular background and physiological importance of most of these changes are yet to be discovered, but it is evident that glycosylation plays an important role in the inflammatory response. Maybe the greatest value of these changes currently lays in their potential diagnostic and prognostic usage, either in combination with current diagnostic markers or on their own. However, determining glycan structures is still technically too complex for most clinical laboratories and further efforts have to be made to develop simple analytical tools to study changes in glycosylation.

Keywords: Glycosylation, inflammatory diseases, serum proteins, rheumatoid arthritis, systemic lupus erythematosus, pancreatitis, sepsis

1. Introduction

Inflammation is a complex biological response of an organism to harmful stimuli, such as pathogens, damaged cells, or irritants. This protective attempt to remove injurious stimuli and to initiate the process of healing is a part of almost all pathological conditions [1]. Both acute and chronic inflammation are highly complex and diverse processes and despite significant efforts invested in studying it, our knowledge how to diagnose, understand and control inflammatory response, is still very limited. Every inflammatory process is accompanied by numerous changes at the site of inflammation as well as many systemic physiological and biochemical changes [2], but in the past two decades more and more attention is being paid to changes in glycosylation [3,4]. Actually, the interaction between selectins and their glycoprotein ligands is

one of the crucial steps for the initiation of inflammation [5], but this has been extensively reviewed elsewhere [6] and is not subject of this review.

Glycosylation is the most diverse post-translational protein modification that provides numerous elaborate ways to modulate protein function [7–9]. Oligosaccharide structures are covalently bound to proteins through nitrogen atom of asparagine or oxygen atoms of serin or threonin side chains, forming N- and O-linked glycoproteins, respectively [10]. N-linked oligosaccharides start with N-acetylglucosamine linked to asparagine and have a glycan core made of five mannose units. They can further differ in branching, to form oligomannose, complex or hybrid types of glycans. Oligomannose type has two to six extra mannoses, while complex type glycans have two or more branches with at least one N-acetylglucosamine and galactose and possibly one sialic acid on each branch. Hybrid type of glycans is a mixture of two types and has one branch of complex structure and one, or more, oligomannose branches (Fig. 1).

Glycans can be present in various structural forms on the same protein, at the same glycosylation site, re-

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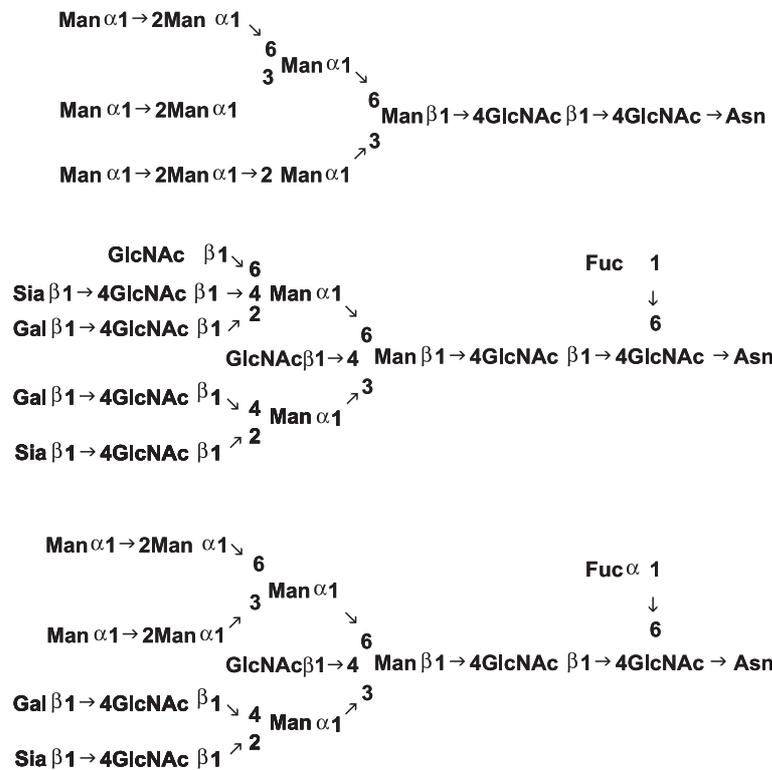


Fig. 1. Examples of oligomannose (top), complex (middle) and hybrid (bottom) glycans. All branches of an oligomannose type glycan end in mannoses. Complex type glycans have two or more branches with at least one N-acetylglucosamine and galactose and possibly one sialic acid on each branch. Hybrid type of glycans is a mixture of two types and has one branch of complex structure and one, or more, oligomannose branches.

sulting in different glycoforms of the same molecule. It is considered that these changes reflect the origin of the molecule, telling about physiological and biochemical condition of an organism at the moment of the release of the exact molecule. The most important feature of glycoproteins is their heterogeneity. It can be expressed from minor to considerable differences through higher branching, loss of monosaccharides from one of glycan branches, through absence or presence of certain monosaccharide such as sialic acid, fucose, N-acetylglucosamine or through type of linkage between sugars.

Many diseases are associated with changes in glycan structures [11]. Since there is no genetic blueprint for glycans, individual glycan structures vary depending on the current level of expression and intracellular localization of biosynthetic enzymes (glycosyltransferases and glycosidases). Consequently, altered glycan structures are often attached to the same protein backbone as a consequence of a pathological processes occurring in a cell that produces the protein. These alterations can be very specific, and studies of serum pro-

tein glycosylation offer a good basis for diagnosis and prognosis of many diseases.

Numerous changes in glycosylation of serum proteins have been reported for inflammatory diseases. Most of these changes are studied in chronic inflammatory conditions, while information on glycosylation in acute inflammation is a bit modest, probably due to the fact that acute inflammation is difficult to catch in its starting point, since this highly dynamic and diverse process includes a cascade of biochemical events and involves many diverse systems. In this review we will present some of the numerous changes in glycosylation of selected serum proteins that were reported to be associated with different inflammatory diseases.

2. $\alpha 1$ -acid glycoprotein

$\alpha 1$ -acid glycoprotein (AGP) is a serum acute phase protein. Its concentration raises several fold during an acute phase reaction like severe burns or trauma, as well as under chronic pathological conditions like

rheumatoid arthritis [12]. It possesses five N-linked complex type glycans [13,14], which may be present as bi-, tri- and tetraantennary structures. Some of these structures may be α 1,3-fucosylated to form a structure known as sialyl Lewis X antigen (Neu5Ac α 2,3Gal β 1,4(Fuc α 1,3) GlcNAc-R).

One of the most interesting features of AGP is that its glycosylation microheterogeneity was found to be altered in many diseases. AGP is a heavily glycosylated serum protein and its both binding and immunomodulatory functions seem to depend on its glycosylation. It represents a good model for glycosylation analysis and is thus, together with IgG, maybe the most studied glycoprotein in different diseases. Among other physiological functions, AGP binds basic and neutral drugs and its binding activity is influenced by ionic interactions involving sialic acid and antennary structures [15].

Changes in biantennary structures of AGP, as well as α 1,3 fucosylated N-glycan structures, have been reported in patients with acute inflammation [16,17] as well as with chronic conditions, such as reumathoid arthritis and diabetes mellitus [18,19]. First findings have mainly been made by using lectin affinity methods through increased binding of Concanavalin A (Con A) [20] and *Aleuria aurantia* lectin (AAL) [21] to AGP in patients with inflammation. It is interesting to mention that studies on different inflammatory diseases showed different AGP microheterogeneity variants [12]. For instance, while a decrease in Con A reactivity was reported in ankylosing spondylitis [22], Con A reactivity was normal in systemic lupus erythematosus [23] and inflammatory lung disease [24], and increased in acute pancreatitis [12]. However, all these findings can vary depending on the type of patients disease activity and presence of concurrent infection. In reumathoid arthritis patients it was shown that the reactivity of AGP with Con A correlates with disease activity [25], while a study on systemic lupus erythematosus showed an increased Con A reactivity in patients with concurrent infection [23]. Studies on chronic and acute infection showed a transition from initially elevated to decreased reactivity to Con A as disease became chronic [26].

Complete glycosylation patterns of AGP can be elucidated only by using more complex methods, such as HPLC (high performance liquid chromatography), HPAEC (high performance anion exchange chromatography), CE (capillary electrophoresis) and MS (mass spectrometry) techniques [17–19], and many findings made by lectin studies have also been confirmed by these methods. Relative increase of Con A reactive

(biantennary) glycoforms of AGP was observed in patients with ulcerative colitis, a chronic inflammatory disease of unknown etiology, even after its remission. In acute inflammation the increase in biantennary structures was found to reach the maximum value in the early phase of inflammation (2nd day after surgical trauma), after which it decreased to control levels [21,27,28]. Kinetics of this change in acute inflammation differs from the variation in the content of fucosylated glycans [21]. Maximum values of fucosylation, reached within the first few days, persisted even 10–30 days after patients were released from the hospital. Since these structures can suppress inflammation, it has been postulated that this increase might have beneficial effects by protecting the organism from overreaction that can occur during inflammation [29]. Since the enzyme responsible for the addition of such fucose is α 1–3 fucosyltransferase, levels of this enzyme are crucial. A study on AGP suggested that inflammatory cytokines regulate the expression of α 1–3 fucosyltransferase VI responsible for α 1–3 fucosylation in liver tissue [17,21] as well as the expression of α 2–3 sialyltransferase required for sialyl-Lewis X formation (since α 2–3-linked sialylation is a prerequisite for α 1–3 fucosylation). Increase in biantennary structures over tri- and tetraantennary ones suggests the increased expression of β 1,4 galactosyltransferase, and the decreased expression of N-acetylglucosaminyltransferases IV and/or V in inflamed hepatocytes.

Work on the prognostic value of α 1-acid glycoprotein glycosylation in septic shock [30], indicated that a modest elevation in biantennary glycans in combination with a strong increase in sialyl-Lewis X was associated with higher mortality than a high transient increase in biantennary glycans with gradually increasing sialyl-Lewis X expression. This clearly demonstrates that the manner of changes in glycan structures can be associated with disease severity.

3. Immunoglobulin G

Immunoglobulin G (IgG) is a glycoprotein with a conserved N-glycosylation site in the Fc region [31,32], and variable glycosylation (either O- or N-linked) in the Fab region [32]. IgG molecules are glycosylated by biantennary complex glycan structures at Asn 297 in the Fc domain that can vary in the presence or absence of sialic acid, galactose, bisecting N-acetylglucosamine or fucose (Fig. 2). Glycosylation of IgG molecules is essential for its binding to all Fc γ receptors (Fc γ R)

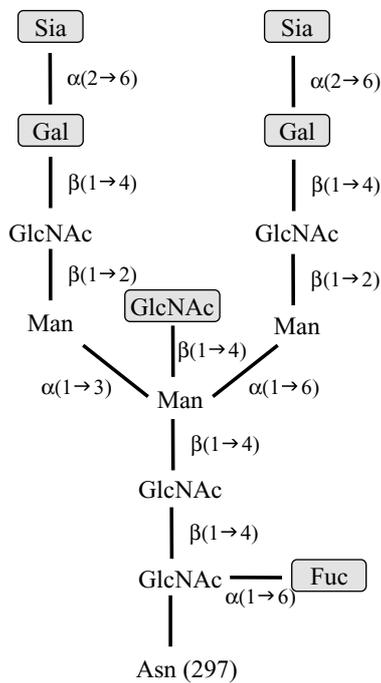


Fig. 2. The largest biantennary complex oligosaccharide found at the Fc region of human serum IgG. There is a common pentasaccharide core containing two mannose (Man) residues attached to a β -mannosyl-di-N-acetylchitobiose unit. Individual glycans can lack one or more of the monosaccharides in striped boxes (sialic acid, galactose, bisecting N-acetylglucosamine, or core fucose). Structure lacking both terminal galactose units is called G0 glycoform and is increased in rheumatoid arthritis and some other diseases.

through maintenance of an open conformation of the two heavy chains [33], and deglycosylated IgG antibodies are unable to mediate *in vivo* triggered inflammatory response [34]. One class of Fc-Fc γ R interactions generates pro-inflammatory effects of immune complexes and cytotoxic antibodies [35]. On the other hand, therapeutic intravenous gamma globulins and their Fc fragments are anti-inflammatory [35–38]. Intravenous gamma globulin is a purified IgG fraction obtained from the pooled serum of healthy donors and is used to treat inflammatory diseases through administration at high doses [39]. When this kind of antibodies was deglycosylated, they were no longer able to mediate anti-inflammatory activity *in vivo* [35]. The same effect can be accomplished by only desialylated intravenous gamma globulin, suggesting that sialic acid could be the sugar that is essential for its anti-inflammatory activity [35].

Among all inflammatory conditions, rheumatoid arthritis is the one where glycosylation of IgG has been studied the most [40–42]. Decreased sialylation and galactosylation of Fc fragments of IgG molecules have

been reported for this chronic inflammatory disease with autoimmune aspects in many studies using different analytic methods [43–45]. It was suggested that different glycoforms of IgG may interact differently with rheumatoid factor auto antibody (RF) in the manner that IgG molecules containing less terminal galactose are preferentially recognized by IgG RF [46]. Since IgG is less galactosylated in rheumatoid arthritis, and glycoforms having 0, 1 or 2 galactose residues (G0, G1 and G2) are usually scanned for rheumatoid arthritis patients, it has been suggested to include quantification of G0 values in routine investigation of rheumatoid arthritis patients and to use G0 levels as prognostic marker for these patients. Very good agreement between different analytical methods (5 different chromatographic protocols and a lectin assay) for measuring G0 values in rheumatoid arthritis patients [41] and the fact that a decrease in galactosylation happens in the very early stage of rheumatoid arthritis provide further support for this suggestions. It is interesting to mention that an increase in G0 glycoforms for more than two standard deviations was shown to have positive predictive value of 80% for rheumatoid arthritis in an individual patient [47]. In the case when patient had both positive rheumatoid factor and increased G0 value this predictive value rose up to 94%. The ratio of G0 was shown to be the best predictive marker for disease course [48] as well as the best marker of joint destruction [49, 50]. Similar changes have been observed in juvenile rheumatoid arthritis [51,52], although lectin study [53] reported no difference between galactosylation of juvenile rheumatoid arthritis patients and the control group until patients were divided in a group of those having acute phase of disease and those in remission. Patients with currently active juvenile rheumatoid arthritis had significantly lower levels of galactose than those in remission, in whom galactose levels were comparable to the control group. Interestingly, fucose levels in both groups were significantly higher than in controls, suggesting that fucosylation, and not only galactosylation of IgG might be an interesting diagnostic marker.

There is a great need to understand whether this altered glycosylation pattern in autoimmune disorders influences antibody-mediated effector functions. Some *in vitro* studies suggested that G0 antibodies gain the capacity to activate the complement pathway via mannose-binding lectin (MBL), which could contribute to antibody-mediated inflammation. Recent study in mice with a genetic deletion of MBL showed that the activity G0 antibodies is unimpaired in these mice [45] and is fully dependent on the presence of activating Fc

receptors. Although this argues against the functional role of interactions between MBL and G0, it should not be neglected that roles of individual glycans (and glycoforms) can be significantly different in mouse and human.

In studies on small vessel vasculitides: Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome, that are characterized by circulating IgG of antineutrophil cytoplasmic antibodies, changes in IgG glycosylation have been reported as well [54]. Similar to rheumatoid arthritis and juvenile rheumatoid arthritis, these conditions are also accompanied by an increased amount of agalactosylated IgG molecules. Since the main pathophysiological model of these diseases is activation of neutrophils by cytokines within the microvasculature, changes in IgG structure could contribute to increased activation of cytokines [54].

In Sjögren syndrome the same changes were observed in a certain subgroup of patients. In this subgroup decreased galactosylation, as well as increased level of bisecting N-acetylglucosamine were found. Patients with normal IgG glycosylation profile had serologically negative RF factor and small risk for developing rheumatoid arthritis, while patients with G0 IgGs and high RF titer had increased risk for rheumatoid arthritis [55].

Glycosylation of IgG was also studied in many other non-rheumatic diseases, mainly malignant states, but since this is not the topic of this paper, changes found in these conditions will not be discussed here. An increase in the proportion of serum IgG molecules possessing an altered Fab glycosylation pattern (designated asymmetric antibodies) was also reported for chronic parasitic diseases [56]. The study on sera of rats in which an acute inflammatory response was produced by subcutaneous inoculation of turpentine oil [56] also showed an alteration in the synthesis and glycosylation of IgG. During acute inflammation there was a decrease in the synthesis of IgG which was not affected by prior oral administration of dexamethasone, however, the turpentine-induced increase in IgG binding to concanavalin A was found to be inhibited upon prior administration of the anti-inflammatory agent.

It is important to stress that changes in galactosylation of IgG are also present in normal, or better to say, physiological conditions. It is very well known that IgG galactosylation is age [57,58] and sex [59] related. It also changes during pregnancy, with reversible increase in galactosylation [60]. Interleukin-6 (IL-6) is often mentioned as one of the possible modulation factors in IgG glycosylation, since activation of IL-6 correlates

with decrease in galactosylation of IgG in animal model [61,62], as well as during pregnancy [63]. However, interleukin-6 is probably just one of the numerous factors contributing to regulation of galactosylation and, in general, glycosylation of IgG as well as other serum proteins.

4. Immunoglobulin A (IgA)

Glycosylation also plays an important role in IgA nephropathy, a form of glomerulonephritis, an inflammation of the glomeruli of the kidney characterized by deposition of the IgA antibody, especially IgA1 subclass, in glomeruli. IgA1 is rich in carbohydrates, carrying N-linked moieties in common with IgG, but also O-linked sugars, which are rare in other serum proteins. Aberrant IgA1 O-linked glycosylation of the IgA1 hinge region is the most consistent finding of all abnormalities of the IgA immune system reported in IgA nephropathy [64,65]. The defect comprises reduced galactosylation of O-linked N-acetylgalactosamine residues with or without changes in the terminal sialylation of the O-linked sugars. These changes have great implications for the pathogenesis of IgA nephropathy, since O-linked sugars lie in an important functional location, close to the ligand recognition site of Fc receptors. Changes in the carbohydrates of IgA1 can therefore affect interactions with receptors and extracellular proteins [66], and lead to IgA immune complex formation and mesangial deposition, which can cause proliferation of mesangial cells and start inflammatory response.

5. Transferrin

Transferrin is a serum glycoprotein whose main role is transport of iron in blood [67]. It is a negative acute phase protein, meaning that its concentration decreases during acute phase response. Decreased transferrin values can also be found in the cases of liver diseases, chronic infections, malnutrition, protein losing enteropathy, trauma or any other severe disease.

Transferrin is made of one polypeptide chain with 679 amino acids and has two N-linked glycan structures on asparagines 413 and 611. Its glycan structures can be biantennary or triantennary and terminate in sialic acids. Changes in branching, fucosylation or sialylation of transferrin have been observed in many malignant [68,69], hereditary (galactosemia [70], CDG [71])

and other severe conditions [72], but in inflammatory diseases as well. Study on transferrin microheterogeneity patterns in sera of nonanemic rheumatoid arthritis patients, iron deficient rheumatoid arthritis patients and patients with the anemia of chronic disease showed increased branching of transferrin glycans in all rheumatoid arthritis groups which correlated to the disease activity (most pronounced in anemia of chronic disease) [73]. Increased branching of transferrin glycans, together with increased sialylation, was also reported for ulcerative colitis patient [74] where changes in glycosylation of α 1-antichymotripsin and α 1-acid glycoprotein were not observed. In this disease glycosylation patterns of transferrin did not differ according to the activity index of ulcerative colitis [74].

The majority of transferrin molecules (85%) in the circulation of healthy individuals are glycosylated with two simple biantennary glycans terminating in α 2,6 linked sialic acids and proper sialylation was found to be important for transferrin function [75–77]. Remaining 15% of transferrin molecules are penta- or trisialylated, while the proportion of less sialylated molecules is negligible [78]. The transferrin of lowered sialylation is called carbohydrate-deficient transferrin, and since its appearance reflect disturbances in glycosylation machinery it is already being routinely used to diagnose chronic alcoholism [79,80] and congenital disorders of glycosylation [71].

Increased serum levels of carbohydrate-deficient transferrin have also been reported in patients with chronic obstructive pulmonary disease, using HPLC [81]. Levels of carbohydrate-deficient transferrin found did not depend on smoking status, although the cigarette smoking is the main risk for chronic obstructive pulmonary disease, and were in inverse correlation with lung function test values, suggesting that defects of glycosylation might be involved in the pathogenic mechanisms behind the development of this chronic pulmonary disease [81].

It was reported that elevated carbohydrate-deficient transferrin predicted prolonged intensive care unit stay in traumatized man [82] and major intercurrent complications were significantly increased in the high carbohydrate-deficient transferrin group (alcohol-withdrawal syndrome, tracheobronchitis, pneumonia, pancreatitis, sepsis, congestive heart failure. . .).

The work on transferrin sialylation in sepsis, using capillary zone electrophoresis, reported decreased sialylation in septic shock patients compared to healthy individuals as well as rapid decrease in sheep model of septic shock [77]. Our recent study in patients with

sepsis indicated that transferrin sialylation decreases rapidly in septic patients (within first 24 hours), but then normalises to the initial values in the next few days [84]. Sialylation of transferrin apparently follows the intensity of inflammatory response and can predict its outcome. It seems that decrease in transferrin sialylation is a part of acute phase response in septic patients and that either more expressed decrease in transferrin sialylation, or no decrease at all, is associated with more severe ways of disease, such as severe sepsis and septic shock.

Different mechanisms of transferrin desialylation were proposed. Regarding the long transferrin half life of 16 days [83], rapid desialylation of transferrin can be explained either by higher activity of neuraminidases, or by higher clearances of highly sialylated transferrin from serum [77]. Same authors speculated that this phenomenon is specific for bacterial infection [77]. However, we observed the same pattern of sialylation changes in the early course of acute pancreatitis [84]. The fact that bacterial infection is not present in early acute pancreatitis argues against this theory, but concerning the role of transferrin as iron transporter [67], the possible physiological role of transferrin desialylation in infection should not be neglected. Bacteria need iron for their metabolism and transferrin is its main source in the host. Since desialylated transferrin has faster clearance from serum and infection is evolutionary the most usual trigger for inflammation, this mechanism probably developed as a part of the inflammatory response, regardless of actual presence or absence of infection.

6. Haptoglobin

Haptoglobin is another serum glycoprotein whose glycosylation was reported to be altered in rheumatoid arthritis [83]. Among these alterations are high levels of abnormally fucosylated forms found in the blood of individuals with active rheumatoid arthritis. These molecules can be detected by using fucose-specific lectin *Lotus tetragonolobus* and discriminate between active and inactive form of disease. However, elevation in fucosylation was not found to be disease specific, since it was also found in sero-negative patients. Elevation in haptoglobin fucosylation, together with higher branching, was also observed in patients with liver diseases initiated by alcohol abuse [86,87]. Alcohol abusers, patients with alcoholic cirrhosis and patients with primary biliary cirrhosis had changed haptoglobin glycosylation pattern, in contrast to patients with chronic active hepatitis [88].

Table 1
Common methods for studying glycosylation changes

Method	Information Obtained	Advantages	Limitations	Conclusion
Lectin affinity methods	presence of specific structure(s)	easy to perform, uses native proteins, inexpensive	provide limited information, detects only some structures	applicable for screening and preliminary tests
Lectin affinity chromatography			unspecific binding of all proteins with certain glycan structure	suitable for separating purified proteins according to glycosylation
Crossed affino-immunoelectrophoresis		great flexibility (could investigate wide range of proteins by using different lectins and antibodies)	cannot handle larger number of samples, semi-quantitative problems with reproducibility	
Electrophoresis + Western blotting + lectin detection	protein separation and lectin detection	insufficient protein separation		
2D electrophoresis + Western blotting + lectin detection		higher resolution of protein separation	multiple steps included	
Enzyme linked lectin assay (ELLA)		fast, high throughput,	limited reliability	suitable for high-throughput screening
Chromatographic methods	type of oligosaccharides, presence of specific monosaccharides, type and number of monosaccharides, sequence and linkages, positions of antennae, anomeric configurations		expensive, require good analytical skills and data interpretation	
HPLC		Quantitative detailed information obtained, possible coupling with other analytical methods	requires purified glycan structures, demanding sample preparation, glycan labelling	provides high amount of information, especially in combination with exoglycosidase sequencing
HPAEC-PAD		no glycan labelling required, quantitative	requires purified glycan structures	good method for either glycan or monosaccharide content analysis
Mass spectrometry	type of oligosaccharides, presence of specific monosaccharide, type and number of monosaccharides, sequence and linkages, antennae positions, anomeric configurations	no glycan labelling required, high sensitivity, high amount of data, reliable	expensive equipment, require good analytical skills and knowledge for data interpretation, limited quantification	

7. α 2-Macroglobulin

Using lectin blots in conjunction with peptide mapping, alpha 2-macroglobulin purified from systemic lupus erythematosus patients was shown to be abnormally glycosylated, suggesting the occurrence of complex glycosylation in this pathological condition [89]. When serum samples of normal donors, systemic lupus erythematosus patients, rheumatoid arthritis patients, a mixed connective tissue disease patient and a Sjogren's syndrome patient were analysed for carbohydrate content of alpha 2-macroglobulin it was not-

ed that the concentration of total monosaccharides on alpha 2-macroglobulin purified from serum samples of systemic lupus erythematosus patients was significantly higher than in normal donors [89]. In the same study was also examined if there are any correlations between the levels of mannose and glucose on alpha 2-macroglobulin and systemic lupus erythematosus. The concentrations of mannose and galactose on alpha 2-macroglobulin from systemic lupus erythematosus patients were significantly higher than from normal donors, in contrast to concentration of glucose where no difference was found between exam-

ined groups. These results suggested that quantification of carbohydrates in selected glycoproteins, such as alpha 2-macroglobulin, may be a novel and alternative clinical marker for systemic lupus erythematosus. Development of an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody directed against abnormally glycosylated serum alpha2-macroglobulin which was capable of recognizing changes of glycosylation in systemic lupus erythematosus patients contributed further to this findings and may be useful in differential diagnosis [90].

8. C-reactive protein

Although C-reactive protein (CRP) is the "most popular" and the oldest molecular marker of acute phase response in both acute and chronic inflammation, work on glycosylation of this serum protein is very scarce. CRP is a pentraxin, calcium binding protein, whose value rises up to 100 times in the first 24 hours as response to initial stimulus, especially bacterial infection. It has been reported that CRPs show variation in both their amino acid sequences and glycosylation patterns in different pathological conditions [91]. These changes in tryptophan contents, together with glycosylation and specific sialylation changes play a contributory role in their binding characteristics (e.g. to antibodies, and other plasma proteins) [92].

Beside the difference in sialic acid content, it's different linkage was found in different diseases, by using *Sambucus nigra agglutinin* (recognizes $\alpha 2,6$ bound sialic acid) and *Maackia amurensis* (recognizes $\alpha 2,3$ bound sialic acid) lectins. CRPs from Visceral Leishmaniasis, tuberculosis and Systemic Lupus Erythematosus contain $\alpha 2,3$ linked sialic acids, while proteins from some other diseases which are not of inflammatory character have $\alpha 2,6$ linked sialic acids [92]. Thus routine use of quantifying CRP may be further supplemented with determination of qualitative alterations of this protein to obtain a unique marker for diagnosis and monitoring of an acute phase response in inflammation.

9. Other and total serum proteins

Many studies done on protein glycosylation in inflammation involved total serum proteins, or combined the glycosylation patterns of few of them. As mentioned before, changes in sialylation of transferrin have been observed in septic patients as well as in ani-

mal model of septic shock [77]. The same study also showed the increase in sialylation of total serum proteins. While following up sialylation of transferrin and total serum proteins during sepsis and acute pancreatitis we also observed an increase in sialylation of total serum proteins that occurred at the early course of disease, followed by normalization afterwards. The most probable explanation for this initial increase is the increased production of highly sialylated acute phase proteins such as $\alpha 1$ -acid glycoprotein [2,93]. Further investigation on sialylation of plasma proteins in inflamed mice showed that the increase in sialylation involves all types of sialic acids ($\alpha 2,3$, $\alpha 2,6$ and $\alpha 2,8$) [94].

Beside the increase in total sialylation, when acute phase response was stimulated in mice by turpentine oil injection, a decrease in total fucosylation was also reported to occur as an early event in the acute phase response [95]. $\alpha 1$ -acid glycoprotein (a positive acute phase protein), $\alpha 1$ -macroglobulin (a non acute phase protein) and $\alpha 1$ -inhibitor3 (a negative acute phase protein) showed similar alterations in sialylation and fucosylation in this case, in contrast to $\alpha 2$ -macroglobulin that contained no significant amount of fucose during acute phase response [95]. These studies demonstrated that changes mentioned happen on pre-existing plasma proteins (since they occur too rapidly for new protein synthesis to have visible effects) and that they also involve non-acute phase proteins. Secretion of sialyltransferases and fucosidases in plasma, reversible endocytosis of proteins or the action of membrane bound enzymes are only some of the possible mechanisms that could explain the observed effects.

Changes of glycosylation of serum proteins have also been detected in psoriatic arthritis where good correlation was observed between total Con A reactivity of serum and serum levels of CRP and IL-6 [96], which was discussed before as a putative regulator of glycosylation pattern of proteins upon inflammation. Increased reactivity to Con A for two serum proteins, AGP and antichymotripsin, was also detected in acute phase response after hip arthroplasty, with no correlation with protein concentrations [97].

Due to development of modern and sensitive techniques for studying glycan structures, the modern approach to study serum protein glycosylation patterns includes first the screening of total serum glycans and then the identification of exact serum proteins responsible for glycan structures involved in changes observed. This approach result in detailed information on glycan structures present in the serum sample and, although it is still time consuming, reveals many differ-

ences. High performance liquid chromatography methods [98–100] and mass spectrometric methods [99,101] are techniques mostly used for this approach and are often further supplemented with different lectin methods (Table 1).

Mass spectrometric approach used to screen glycan structures in cirrhotic patients [101] showed an increase in bisecting N-acetylglucosamine and core fucose, and revealed the presence of an important population of neutral oligosaccharides in this disease. HPLC analysis of N-glycans from diabetic mice and humans with type 2 diabetes showed an increase in alpha1,6-fucosylation [100], which was more expressed in mice. Study of total glycosylation profile of serum proteins in sepsis and acute pancreatitis also revealed many changes in these diseases as well as through their courses [102]. Glycans from these patients were analysed by normal phase and weak anion exchange HPLC, exoglycosidase digestions and mass spectrometry. Increase in the amount of fucosylated trisialylated structures and tetrasialylated structures, increase in ratio of outer arm to core fucose, changes in levels of mannose structures and in the degree of branching are just the most prominent changes found. The relative proportions of different glycans changed daily in studied patients and some differences were also observed between sepsis and pancreatitis, probably reflecting that in these two conditions the acute phase response is triggered by a different stimulus which is associated with different patterns of cytokines' production.

10. Conclusions

In this review we described some of the glycosylation changes found in different inflammatory conditions on main serum proteins. Molecular background for these changes and their role in pathophysiological processes are yet to be discovered, but it is evident that glycosylation plays an important role in inflammatory response. Maybe the greatest value of these changes currently lays in their potential diagnostic usage, either in combination with current diagnostic markers or on their own. Studying changes in glycan structures can help to early diagnose pathological conditions and in some cases also predict course of the disease. However, determining glycan structures is still technically too complex for most clinical laboratories and further efforts have to be made to develop simple analytical tools to study changes in glycosylation.

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