Chapter

The Structure of Leukocyte Sialic Acid-Containing Membrane Glycoconjugates is a Differential Indicator of the Development of Diabetic Complications

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Abstract

Glycans, as potential prognostic biomarkers, deserve attention in clinical glycomics for diseases diagnosis. The variety of glycan chains, attached to proteins and lipids, makes it possible to form unique glycoconjugates with a wide range of cellular functions. Under leukocyte-endothelial interaction, not only the availability of glycoconjugates with sialic acids at the terminal position of glycans are informative, but also the type of glycosidic bond by which sialic acids links to subterminal carbohydrates in structure of glycans. The process of sialylation of leukocyte glycoconjugates undergoes considerable changes in type 1 diabetes mellitus. At early stage of disease without diabetic complications, the pathology is accompanied by the increase of α2,6-linked sialic acids. The quantity of sialic acid-containing glycoconjugates on leukocytes surface increases in condition of disease duration up to five years. However, the quantity of sialic acids linked by α2,6-glycosidic bonds decreases in patients with the disease duration over ten years. Therefore, sialoglycans as marker molecules determine the leukocyte function in patients with type 1 diabetes mellitus, depending on the disease duration. Changes in the glycans structure of membrane glycoconjugates of leukocytes allow understanding the mechanism of diabetic complications development.

Keywords: sialic acid, glycans, glycoconjugates, sialyltransferases, sialidases, leukocytes, type 1 diabetes mellitus

1. Introduction

Glycans, as potential biomarkers of health and illness, deserve attention in clinical glycomics for early-stage disease diagnosis [1, 2]. It is not surprisingly that abnormal (aberrant) glycosylation of proteins and lipids have been observed in many diseases, including cancer, cardiovascular disease, immune deficiencies and diabetes [3].

Diabetes mellitus (DM) is one of the most common endocrine diseases that have been identified as one of the priority issues for national health systems around the world. Type 1 DM is characterized by a progressive autoimmune destruction

of pancreatic β-cells, leading to insulin deficiency and chronic hyperglycemia [4]. The social significance of this problem is that DM associated with development of numerous concomitant diseases, early disability, and metabolic complications [5]. Insufficient control of glucose level in blood may increased the risk of microvascular (nephropathy, retinopathy, neuropathy) and macrovascular (peripheral artery disease, coronary artery diseases, congestive heart failure, myocardial infarction, stroke) complications. In addition, individuals with DM have increased risk of physical and cognitive disability, depression and cancer [6]. The various complications related to diabetes are determined by changes of blood components. Blood cells (leukocytes, erythrocytes and thrombocytes) are of particular interest because they are directly exposed to high glucose concentrations [7]. Blood cells aggregate strongly to the vessel wall and adhere to each other, which leads to the development of pathological changes in capillary blood flow and microcirculation disorders in diabetes [4–6].

Leukocytes are the major cells of the inflammatory and immune response that defends against different type of infection, consequently these cells are important object for investigation [5]. The clinical and experimental studies of human blood in case of DM and animals with streptozotocin-induced diabetes demonstrate significant violations of the morphofunctional state of leukocytes. The dysfunction of chemotaxis capacity, adhesion and migration, reduction of phagocytic activity and bactericidal ability of leukocytes correlate with the level of hyperglycemia in blood [8, 9].

The impairment in the functions of immunocompetent cells leads to a decrease in immune defense and the development of chronic infectious/inflammatory processes in organism of people with type 1 diabetes [10]. Chronic inflammation is the main cause of progression of diabetic complications which leads to dysfunction of the extremities, retina, kidneys, nerves, heart and blood vessels. According to statistics, most of patients die from angiopathic complications of diabetes. Screening of complications provides with possibility to reduce the risk for their development and progression [11]. Therefore, expansion of diagnostic methods for characterization of changes in the morphofunctional state of leukocytes and the search for preventive remedies that would ameliorate the clinical condition of patients is a relevant problem today.

2. Importance of membrane glycoconjugates in providing the functional activity of leukocytes

Experimental studies have shown that cells of the immune system are exposed to the direct and indirect effects of high blood glucose concentrations in patients with diabetes [4, 5]. Glucose metabolism pathways are activated under conditions of hyperglycemia include the autooxidation of glucose, caused glycation of long-lived proteins; the hexosamine pathway, which leads to the glycosylation of hydroxyl-containing amino acid residues; sorbitol metabolism, accompanied by the formation of free radical; and oxidative phosphorylation leading to mitochondria electron transport chain intensification and the generation of superoxide-anion radicals [12, 13]. Glucose autooxidation and glycation of proteins and lipids leads to an accumulation of advanced oxidation protein products and advanced glycation end products (AGEs), which are difficult to eliminate from the blood and remain in circulation [14]. They are also the source of reactive oxygen species (ROS) since they imitate metal containing oxidation systems. Excessive formation of ROS and reactive nitrogen species (RNS) leads to the development of oxidative-nitrative

stress [15]. These changes create a favorable background for the formation of micro- and macrovascular diabetic complications [15, 16].

In condition of hyperglycemia, leukocytes are preactivated by ROS and RNS, angiotensin II, and AGEs. The interaction of AGEs with their receptors, RAGE, causes intracellular signal transduction, which leads to changes in gene expression, overproduction of free radicals, the release of pro-inflammatory molecules (tumor necrosis factor α (TNFα), interleukin 1β (IL-1β), IL-2, IL-6 etc.), and changes in the activity of intracellular enzymes [17].

Glycosyltransferases and glycosidases, which involved in the synthesis of glycans of glycoconjugates, pay much interest among cellular enzymes [18]. In general, mammalian glycans are the product of several types of glycohydrolases and dozens of glycosyltransferases, which act sequentially in the process of oligosaccharide chains synthesis. Each of the glycosyltransferases uses one type of sugar substrate and forms a specific bond between one monosaccharide and a glycan precursor. Thus, the set of glycosyltransferases in the cell determines what type of glycans, among the large number of possible structures, will be formed [19].

The variety of monosaccharides is very large, but most often the carbohydrate components of glycoconjugates of eukaryotic cells include glucose (Glc), N-acetylglucose (GlcNAc), galactose (Gal), N-acetylgalactose (GalNAc), mannose (Man), N-acetylneuraminic acid (Neu5Ac), also known as sialic acid (Sia). Different monosaccharides, combined in a specific sequence by glycosyltransferases, form a glycan, which at one end attaches to a protein or lipid molecule. The formed glycoconjugates are the main macromolecular constituents of biomembranes [19, 20].

The diversity of glycans attached to proteins and lipids makes it possible to form unique glycoconjugates with a wide range of cellular functions. Glycoconjugates play an important role in various biological processes, in particular, glucose homeostasis, protein quality control, cellular differentiation, adhesion, intercellular signaling and inflammation. It is known that carbohydrate residues increase the solubility of glycoproteins, protect against proteolysis, influence on their folding, intracellular transport and secretion. Glycoconjugates are components of the glycocalyx, providing specific interactions with ligands, intercellular contacts and communication. Glycans of glycoconjugate are involved in the formation of the immune response, blood clotting and provide the individuality of organisms and their plasticity [20].

The immune system is highly controlled and fine-tuned by glycosylation, through the addition of a variety of glycans to virtually all adhesion molecules and receptors of leukocytes. Glycoconjugates are implicated in fundamental cellular and molecular processes. Glycans perform function of molecular recognition that regulates both stimulatory and inhibitory immune pathways [21]. The presence of modified carbohydrate determinants in the glycan structure modifies the biological activity of the entire glycoconjugate. The interaction of specific ligand with its modified receptor leads to violations at the level of transmembrane and intracellular signaling [22]. In according to the importance of glycans in the immune system, scientific researches emphasize the essential contributions of glycosylation in the regulation of innate and adaptive immune responses [21]. Therefore, today the scientists (biochemists, molecular biologists, immunologists, pathologists and pharmacologists) are making the great efforts to explore the interrelations of carbohydrate determinants with their glycobiology. Establishing changes in the glycans structure of membrane glycoconjugates of immunocompetent cells makes it possible to understand the mechanism of pathological changes in condition of diabetes and diabetic complications.

Thus, changes in intracellular metabolism, intensification of glycation processes and the development of oxidative-nitrative stress in blood cells under conditions of prolonged hyperglycemia are the main factors that induce pathological changes in the structure of their components and affect their functional state [12, 20].

3. Sialoglycoconjugates of leukocytes as the main regulators of molecular and cellular interactions

Glycoconjugates of leukocytes contain sialic acid as the terminal sugar and play important roles in many physiological processes. Sialic acid normally exists in the periphery of non-reducing end of the oligosaccharide chains of many glycoproteins and glycolipids. They are involved in carbohydrate-protein interactions during cell recognition, in cell–cell interactions involving functional receptors, in the binding of pathogens such as viruses, bacteria or parasites [19, 23]. Sialic acids are also implicated in the processes of activation, differentiation, survival and apoptosis of leukocytes. Sialoglycoconjugates affects cellular adhesiveness, antigenicity, action of some hormones, catalytic properties of enzymes, modulating the affinity of cell surface receptors and transmembrane signaling [19, 20]. It is obvious that sialic acids are important molecular determinants of many immune processes. To implement these functions, organisms have a range of proteins (sialospecific lectins) that recognize surface-exposed sialic acids in glycoconjugates [19].

The variety of functions indicates the importance of sialic acid in cell biology. The biology of sialic acids should be considered from the point of view of their dual function. On the one hand, sialic acid acts as biological mask agent by masking recognition sites such as receptor molecules of cell membranes. On the other hand, sialic acid plays a role as recognizable cell patterns. Sialic acids as ligands are recognized by lectins, antibodies, hormones or as receptors recognize extracellular markers in the molecular processes of cell interactions [23–25]. Activation of cells can lead to the opening of ligand-binding sites with a subsequent increase in binding affinity, lowering the cellular activation threshold, or removal of inhibitory signals [26, 27].

Sialic acids can participate directly or indirectly in multiple cellular events and overall immune response [28]. Sialic acids contribute to cells being "self" and, thus, weakens immunoreactivity. That is why they are not recognized by immune system cells or macrophage lectins. The loss of these masking monosaccharides makes the cell "foreign", activating the body's immunoreactive response. Therefore, sialic acids can be considered components of innate immune protection [29]. These acids have recently been recognized as being involved in most important phenomena of molecular and cellular interactions in immune regulation [30, 31]. In this respect, sialic acids have been associated with inflammatory diseases, malignancies, cardiovascular disease and diabetes [32].

Sialic acids are group of monosaccharides with high structural diversity, which are chemically derived from nine carbon acidic sugars – neuraminic acids. The most abundant member of the family carries an acetyl moiety linked to the amino group of fifth carbon (C5) giving the Neu5Ac. A feature of its structure is the presence of a carboxyl group near C1, which determines the negative charge of the molecule at physiological pH and characterizes it as a strong organic acid (pK 2.2). More than fifty derivatives of neuraminic acids have been found in nature. The most common sialic acid derivatives found in mammals are Neu5Ac and N-glycolylneuraminic acid (Neu5Gc), whereas in humans Neu5Ac is the dominant sialic acid [19, 23, 33].

Due to their negative charge at physiological pH and hydrophilic property sialic acids stabilize conformation of molecules, can impact protein oligomerization, the

interactions of proteins with other proteins and the extracellular matrix. Sialic acids as an essential compound of all cell membranes play an important role in maintaining the structure, permeability and integrity of the cell membrane [28, 34]. Not surprisingly, sialic acid exponation is dynamic, changes during development and is altered in numerous diseases [35]. Changes in sialylation are associated with oxidative stress induced by several disorders including diabetes. It has been proven that level of sialic acid increased in plasma in condition of inflammatory processes and DM [36]. The relation between sialic acid and diabetes duration most likely follows from the association sialic acid with microvascular complications, which are well established to be related to glycemic control [37]. Therefore, sialic acid concentrations in the blood may be a useful marker of the development of diabetic complications, but there have been no many studies examining the link between sialoglycoconjugates and complications in type 1 DM.

The structural diversity of sialoglycoconjugates is due not only to the diversity of derivatives of sialic acids in their composition, but also depends on the type of glycosidic linkages (2,3, 2,6, 2,8, and 2,9) with subterminal sugars. The sialylation of oligosaccharide chains of glycoconjugates is carried out with the participation of the family of enzymes sialyltransferases (STs). About 20 STs have been characterized [19]. STs are divided in four main subfamilies, namely the ST3Gal, ST6Gal, ST6GalNAc and ST8Sia, depending on the glycosidic linkage formed and the monosaccharide acceptor recognized [35, 38]. ST3Gal, ST6Gal, ST6GalNAc and ST8Sia link Neu5Ac via its C2 to the C3, C6 positions of other carbohydrates or the C8, C9 positions of another sialic acids, generating α2,3-, α2,6-, α2,8, or α2,9-linked sialic acids, respectively [19, 39]. Sialyltransferase-mediated addition of sialic acid on glycans usually stops their further growth and modifies charge, steric hindrance, conformation and flexibility, underlying the importance of STs in shaping the structures and functions of sialoglycans [35, 40].

In the structure of leukocytes' glycans sialic acids are frequently the terminal residues of glycans and are mostly attached either by a 2,3- or 2,6-glycosidic bond to Gal or GalNAc of oligosaccharide chains [19]. The ST6Gal and ST6GalNAc, which are present in leukocytes, catalyze the transfer of Neu5Ac from CMP-Sia (cytidine-5′-monophospho-N-acetylneuraminic acid) to the C6 hydroxyl group of a terminal Gal or GalNAc residues, respectively, with the formation of α2,6-linkaged sialic acids in the oligosaccharide chains of glycans [19, 20, 38]. The ST3Gal comprises family with six members (ST3Gal I–VI). The expression of ST6Gal-I is tissue specific and regulated by multiple transcriptional promoters [41, 42]. An inducible and liver-specific promoter drive high ST6Gal-I expression during inflammation with increase in secreted ST6Gal-I in blood [43]. Activated platelets release the CMP-Sia that serves as the donor for circulating ST6Gal-I, allowing for the remodeling of the glycans of hematopoietic stem cells and multipotent progenitors (HSC/MPPs) [44]. Thus, inducible promoter is important for regulation of hematopoiesis [45]. The ST3Gal-V add a sialic acid to terminal Gal residues with the formation of α2,3-glycosidic linkage, while ST3Gal-IV sialylates Galβ1,3GalNAc terminated structures in glycoconjugates and Galβ1,4(3)GlcNAc structures found on N- and O-glycans [46]. The ST3Gal-IV and ST3Gal-VI involved in the synthesis of the sialyl Lewis^X (sLe^X) determinant on leukocyte E-, L- and P-selectin ligands [19, 46]. Leukocytes express a number of different selectin ligands, including E-selectin ligand-1, P-selectin glycoprotein ligand-1, CD43, CD44, β2-integrins, ets. [35, 47].

Glycosylation of cell-surface structures of leukocytes is important in the accomplishment of the immune function by these cells in organism. The membrane structures of leukocytes are decisive in the processes of extravasation, the migration of leukocytes from blood vessels into the extracellular space. In order to penetrate the vascular wall, leukocytes initially interact with the endothelium,

roll over its surface, undergo dense adhesion, dissolve, and finally move through or between endothelial cells of the blood vessel [48–50]. Leukocyte chemotaxis depends on the surface sLe^X and E-selectin of vascular endothelial cells. E-, L- and P-selectins are exposed by endothelial cells, leukocytes and platelets, respectively. Selectins are carbohydrate-binding proteins that recognize the sLe^X structure (Neu5Acα2,3Galβ1,4(Fucα1,3)GlcNAcβ-R), capping N- and O-glycans as specific ligands [51–53]. These sialic acid-containing moieties are required for leukocyte binding to selectins on endothelial cells and their rolling [54, 55]. Combinatorial knockout of ST3Gal-IV and ST3Gal-VI that are the involved in sLe^X synthesis leds to a decrease in neutrophil binding to E- and P-selectins, selectin-dependent rolling, and lymphocyte homing [46]. The selectin profile of cells can change under the influence of cytokines in case of development of inflammatory process, infection or under the influence of ROS. In tissue inflammation, cytokines stimulated endothelial cell production of E-selectin, which could recognize sLe^X on the leukocyte surface and bind it, promoting leukocyte adhesion to the vascular endothelium and, subsequently, to the inflammatory tissue or locations of injury [32, 56]. Therefore, the recognition of all types of selectins is mediated with sialic acid residues [19].

In the catabolism of sialoglycans of glycoconjugate involved extracellular and intracellular sialidases, a glycoside hydrolase, that specifically hydrolyze release α-linked sialic acid residues through hydrolysis of the glycosidic bond between the acidic sugar(s) and the internal acceptor. Four different sialidases (also termed as neuraminidases – NEUs) in mammalian cells, NEU1, NEU2, NEU3 and NEU4, have been described [57]. These NEUs exhibit differences in cellular localization, substrate specificities, physiological functions and expression patterns in different tissues and physio/pathological conditions [35, 57, 58]. The NEU1 is found in the lysosome and on the cell surface and is the most highly expressed of this sialidase family [35]. The level of NEU2 is extremely low and the content of NEU3 and NEU4 are about 10% of NEU1 in tissue separately [57]. The lysosomal sialidase NEU1 initiates the degradation of sialoglycoconjugates [59]. The NEU1 is capable of hydrolyzing a wide range of glycoproteins, oligosaccharides and ganglioside near neutral pH. It exclusively acts on glycoproteins and preferentially cleaves α2,3-linkages over α2,6- or α2,8-linkages [19, 35]. In addition, NEU1 may have extralysosomal localization and focus on the periphery of activated lymphocytes. The NEU1 controls several aspects of the immune response by the desialylation of molecules, such as Toll-like receptor 4 and adhesion molecules involved in the recruitment of leukocytes to inflammatory sites [35, 57]. Desialylation of sialyl α2,3-linked Gal residues of Toll-like receptor 4 is essential for receptor activation and cellular signaling [60]. The cytosolic sialidase (NEU2) can hydrolyze sialic acids from glycoproteins and gangliosides [61]. The plasma membrane-associated sialidase (NEU3) is a key enzyme for ganglioside hydrolysis [57]. The NEU4 is localizing in the lysosomal lumen or bound to the outer mitochondrial membranes via protein–protein interactions or the ER membrane-associated. Its exhibits the highest activity with gangliosides as well as sLe^X and sLe^a antigens [35, 57, 58]. Sialic acid is actively exfoliated from the cell surface by extracellular sialidases during leukocyte activation. This process plays an important regulatory role in cell activation and differentiation [62].

Metabolism of sialic acids includes the cooperation of enzymes that catalyze the biosynthesis, activation, transfer of sialic acids to glycoconjugates, as well as the removal and degradation of sialic acids [63]. The aberrant expression of STs and NEUs accelerates and sustains sialylation status on glycoconjugates [64]. Therefore, knowledge in this field of glycobiology allows to predict biological events in case of increase or decrease in the amount of sialoglycoconjugates on the cell surface or under conditions of modification or structural changes of these acids in certain

types of cells. Thus, STs, NUEs and sialic acids itself represent important therapeutic targets for medicinal chemistry and biopharmaceutical industry [65, 66].

4. Lectins as diagnostic molecular probes for determining the glycosylation profile and structural changes of glycans

Glycocode information is read in living organisms with the help of specific compounds – lectins. Lectins are sugar-binding proteins that can specifically recognize glycans of glycoconjugates without disrupting the structure of the recognizable carbohydrate-containing ligands.

Since surface glycoconjugates have a unique structure for each cell type, they can be identified, quantified and characterised structural changes in glycans using specific lectins. Nowadays, lectins, their properties, the importance of these proteins in the life of organisms and their applying in experimental biology and medicine are the subject of research in the world's science laboratories. Lectins excluded from living objects are valuable biochemical reagents that are used in experimental cytochemistry, in the diagnosis of some diseases, and in biotechnology for isolating certain carbohydrate-containing molecules [20, 67].

Interactions of sialic acids with lectins play a leading role in many physiological and pathological processes. Therefore, sialospecific lectins are used to recognize sialic acids with specific linkages to subterminal sugars. Wheat germ lectin (WGA) specifically binds to β,DGlcNAc and Neu5Ac. The *Maackia amurensis* lectin (MAA) and *Sambucus nigra* lectin (SNA) are commonly used to recognize the α2,3-linked (Neu5Acα2,3Gal) and α2,6-linked (Neu5Acα2,6Gal/GalNAc) sialic acid residues, respectively [20, 68, 69]. Sialospecific lectins apply in lectin microarray [70], histochemistry [71], in lectin blot [72, 73], fluorescent image and flow cytometry [74] (**Figure 1**). At the same time, the combination of lectins with monoclonal antibodies can be used to obtain complete information on the antigenic repertoire of cells both in normal and in case of pathologies [73].

Blood leukocytes are similar in structural organization, and, at the same time, they differ significantly in biochemical structure. It is very important to understand the morphofunctional state of the cell is to be able to detect these differences. Numerous methods are used for this purpose [70]. Aggregatometry is one of the assays used to evaluate the functional properties of platelets, leukocytes and erythrocytes in the dynamics, monitor antiplatelet therapy, study the mechanisms of aggregation. The aggregation capacity of cells is assessed by such parameters as the degree, rate and time of aggregation [20].

The substances of protein (lectins, proteolytic enzymes, chemoactive peptides); lipid (metabolites of arachidonic acid, liposomes); carbohydrate (heparin, dextransulfates) or other nature (phorbol esters, amphotericin B, ADP, organic dyes – alcyanine blue, ruthenium red) can be inducers of aggregation. Lectins used in aggregatometry are divided into lectins-mitogens (ConA, PHA) and polyvalent lectins (WGA, SNA). Polyvalent lectins have two or more binding centers of carbohydrate determinants (carbohydrate-recognition domains) on the cell surface. The aggregation of cells by such lectins is due to the formation of intercellular molecular bridges. The ability of each subunit of lectin to bind sugars individually leads to the formation of a cross-linked structure of the aggregate. The efficiency of lectin-induced aggregation is determined by the processes of clustering of lectin receptors on the cell surface [20, 75].

The aggregation capacity of leukocytes is studied to model their pre-migratory state before leaving the bloodstream, i.e. before diapedesis, or to analyze phagocytic activity. It is consider that phagocytosis involving lectin-carbohydrate interactions

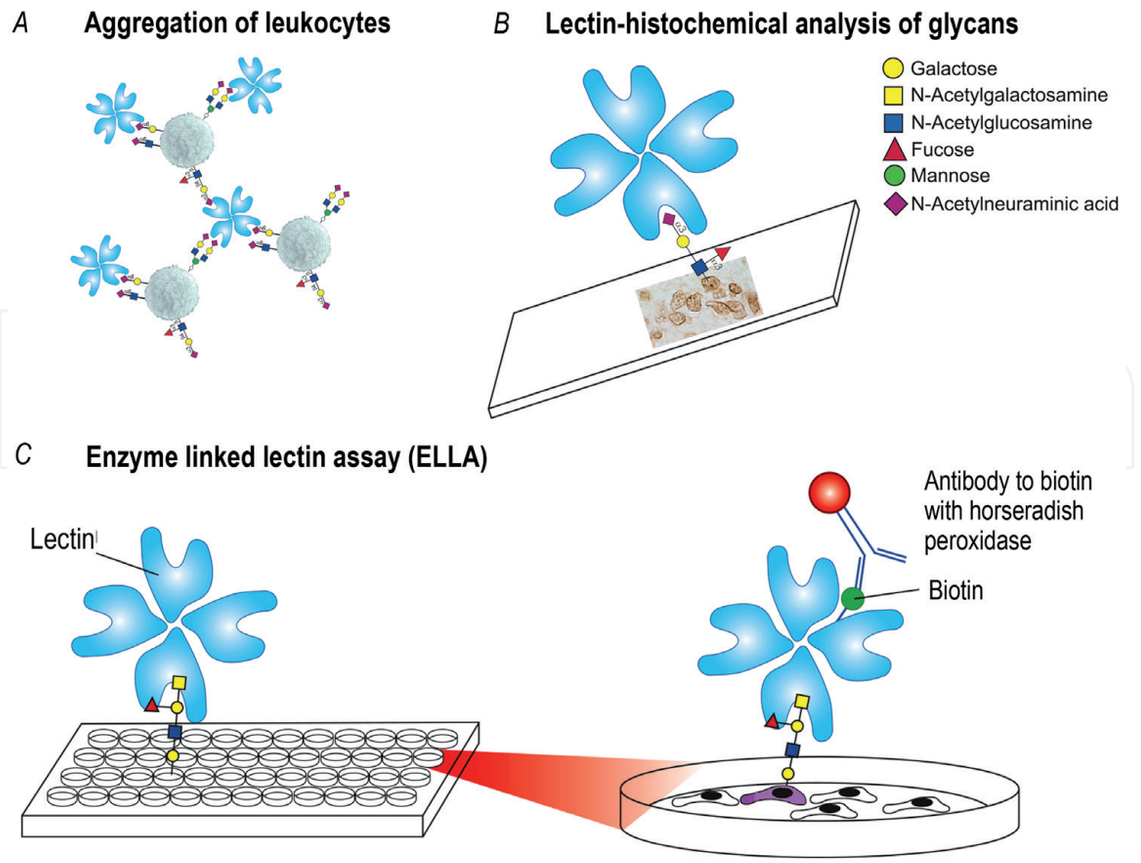

Figure 1.
Examples of uses of lectins in glycobiology. Many plant and animal lectins are multivalent. In particular, the lectin is shown with four carbohydrate binding domains. (A) Lectins bind of surface glycoconjugates of leukocytes, causes cell aggregation. (B) Histochemical analysis of surface glycans. (C) Enzyme linked lectin assay: biotinylated lectins bind to glycoconjugates on the surface of cells immobilized to the bottom of the well of a flat-bottomed plate; bound lectins are detected by antibodies to biotin with horseradish peroxidase.

is one of the oldest evolutionary forms of this process [76]. Phagocytosis during evolution was significantly displaced by antigen–antibody interactions, but did not lose importance in the formation of a nonspecific immune response [77].

5. Changes of carbohydrate determinants of glycoconjugate mediate the functional state of leukocyte in type 1 DM

Leukocytes are markers of the immune homeostasis and receive signals from the microenvironment through the glycans of receptors. Lectins of certain carbohydrate specificity are ligands that selectively activate chemokine receptors. The response of cells to lectins *in vitro* makes it possible to analyze the chemical structure of the carbohydrate determinants of glycoconjugates on the membrane of leukocytes [66, 78].

Lectins WGA, SNA and MAA, which specific to sialic acids, are used to determination sialylated glycoconjugates and differentiation various types of sialic acid links with subterminal carbohydrates of glycans (SNA recognizes α2,6-links, while MAA identifies α2,3-links, **Figure 2**) [75, 79].

Alteration of the amount of sialic acids on the surface of leukocytes is an additional level of regulation of cells affinity to signaling molecules (cytokines, hormones), pathogenic microorganisms and determines the nature of cell–cell interactions [20].

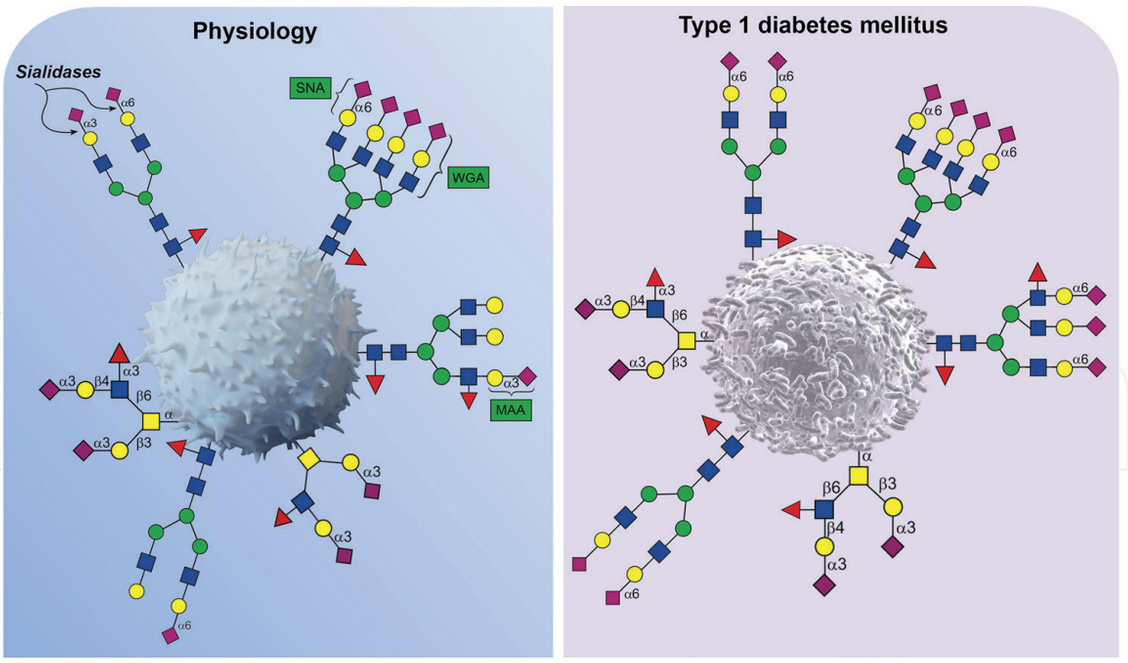

Figure 2.
The structure of leukocyte sialic acid-containing membrane glycans in physiological state of cells and in type 1 diabetes. Sialic acids, depending on the type of glycosidic bond in the structure of the glycan, are recognized by WGA, SNA and MAA lectins.

The most significant changes of increasing lectin-induced aggregation of leukocytes in type 1 diabetes have been observed using lectin WGA. An increase in the degree and rate of WGA-induced aggregation of neutrophils in diabetes is a sign of increased of N-acetyl-β,D-glucosamine-containing and sialic acid-containing glycoconjugates on surface of leukocytes [80]. This indicates that synthesis of hybrid types of N-glycans is occured by activated N-acetylglucosaminyltransferase-III (GnT-III) and incomplete glycosylation of proteins and lipids [20]. As a result, glycoconjugates with terminal β,D-GlcNAc residues are exhibited on the leukocyte surface and determined high rates of WGA-induced aggregation [81].

The structural characterization of neutrophils glycoconjugates showed that cell surface N-glycans are highly sialylated, and many of their "antenna" play an important role in selectin-mediated neutrophil circulation [82]. Glycome of neutrophils is consisted mainly of complex bi- tri- and tetra-antennary N-glycans (**Figure 2**). Their antennae are predominantly terminated with Neu5Ac and Le^X (Galβ1,4(Fucα1,3)GlcNAc) epitopes [83]. The ST3Gal-IV knockout results in significant reduction in the synthesis of sLe^X structures in neutrophils. These cells show significant impairment in rolling and adhesion to the endothelial cells [84]. All these structural changes in the carbohydrate chains of glycoconjugates of leukocytes induce disturbances of molecular signals perception from the microenvironment, affecting interaction of leukocytes with other circulating blood cells and vascular endothelium in condition of diabetes [7, 20, 85].

Sialic acids can mask, i.e. change the structure of carbohydrate components of various specific receptors on the cell surface [19]. There is the receptor to N-formyl-methionyl-leucil-phenylalanine, C5a component of the complement system, IL-8, the receptor of granulocyte-monocyte colony-stimulating factor and the cell receptor 3 (Mac-1) among WGA-binding glycoproteins. The interaction of neutrophils with the intercellular adhesion molecule 1 (ICAM-1, CD54), which is involved in the adhesion of leukocytes to the vascular endothelium occurs via the Mac-1 receptor. On the other hand, WGA-specific receptors are involved in the

stimulation of respiratory burst in neutrophils by activating NADPH oxidase and followed formation of ROS [86–88].

The content of GlcNAc and Neu5Ac residues in glycans of glycoconjugates of the plasma membrane of neutrophils increases in type 1 diabetes [72]. It may be one of the main causes of nonspecific damage of tissues and cells, which are close to stimulated neutrophils. Under such conditions, neutrophils produce ROS and cause erythrocytes, platelets, fibroblasts and endotheliocytes death, inactivate enzymes, lead to changes in the structure of proteins and lipid peroxidation [6, 20].

Interaction of glycoconjugates of polymorphonuclear leukocytes with lectin SNA changes significantly under DM [80]. The level, velocity and time required for the maximum neutrophilic granulocyte aggregation in patients with type 1 DM duration of up to 5 years have been different from these indicators in patients with diabetes lasting more than 10 years. In particular, in the early stages of the disease, the degree of neutrophils aggregation, as well as the rate of SNA-induced aggregation have been four times higher than in patients with the disease over ten years [20, 80]. It is assumed that with the disease progresses, changes in leukocytes are associated with neutrophil subactivation processes that lead to the release of granule contents into the extracellular space, especially intravascularly. Degranulation leads to the lowering of cells aggregation [88, 89]. It is known that elevated glucose levels inside the cell have an inhibitory effect on a number of enzymes that are involved in the biosynthesis of the oligosaccharide chain of glycans. One of such enzymes is STs, which catalyze the attachment of sialic acid to the terminal sugar in glycan structure [19, 39]. Hyperglycemia is probably one of the factors that mediates the glycan profile violation of leukocytes in diabetes.

Decreased aggregation of neutrophils in patients with DM under the addition of MAA lectin indicates the presence sialic acids in the structure of glycoconjugates of neutrophil membranes in a small amount. These α2,3-linked sialic acids affect both the dynamic and kinetic parameters of the neutrophil aggregation process [72, 90]. The decrease in sialic acid content in the cellular glycocalyx is most often due to the enhanced desialylation of the membrane glycoconjugate. It is worth noting that sialic acids which are linked to subterminal sugars of the glycoconjugates oligosaccharide chains by the α2,3-glycoside bond are much more likely to undergo hydrolytic cleavage by sialidases than α2,6-linked residues of these sugars [90]. Cleavage of sialylated oligosaccharide fragments from glycoconjugates or exfoliation of the whole molecules of sialoglycoconjugates can be another reason of loss of sialic acids from the cell surface. However, there is often a combination of all these factors [20, 81]. Decreased α2,3-linked sialic acid on the surface of leukocytes leads to impaired perception of signals from the extracellular space, interaction with other cells, as well as numerous bacteria, protozoa and viruses. Desialylation of surface glycoconjugates of polymorphonuclear leukocytes leads to increasing of their adhesive properties, which promotes the migration of neutrophils through the vascular endothelium [91].

The interaction of glycoconjugates of mononuclear leukocytes with lectin MAA, which reacts with Neu5Acα2,3 Gal/GalNAc terminal endings glycan, have been markedly inhibited under diabetes [90, 92]. It has been found that the decrease in sialic acid content usually occurs due to increased activity of endogenous sialidases in activated T cells and monocytes [46]. This leads to increased production of cytokines by lymphocytes and interaction of monocytes with hyaluronic acid – a component of extracellular matrix [93, 94]. The NEU1 and NEU3 are expressed in monocytes in the process of their differentiation into macrophages. Desialylation of glycans on the surface of monocytes by exogenous NEU resulted in activation of ERK1/2 and p38 MARK signaling pathways and increased production of IL-6, IL-1β, MIP-1α and MIP-1β [94, 95]. Pro-inflammatory cytokines cause endothelial

dysfunction by increasing capillary permeability, inducing prothrombotic properties, promoting leukocyte recruitment by synthesis of adhesion molecules and chemoattractants, and play a role in macroangiopathy by promoting dyslipidemia. Thus, it is unlikely that the increased circulation of sialic acid is the result of desialylation of glycoconjugates. However, there is evidence that sialic acid is reduced in endothelium and erythrocytes in diabetes, which may be important in the pathophysiology of vascular disease [37].

Due to fact that terminal α2,3-linked sialic acids are included, in particular, in the structure of the CD45 receptor, which mediated an increasing of T cell proliferation [96], the decrease content of sialic acids in type 1 diabetes indicates a violation of this function in immunocompetent blood cells. Studies showed that the sialylation of T cell CD45 by ST6Gal-I blocks galectin-1 clustering of CD45 and resulting cell death [97]. The α2,6-sialylation of FasR blocks binding of Fas-associated adaptor molecule to the FasR death domain, thus inhibiting the formation of the death-inducing signaling complex [98].

Lectins SNA and MAA interact with $CD45^+$ leukocytes [96]. CD45 is a transmembrane glycoprotein found on T, B, NK cells, granulocytes, and monocytes. It has a cytoplasmic tail with cytosolic phosphotyrosine phosphatase activity. CD45 is the antagonist of tyrosine kinase of insulin receptor, whereas it can show high activity towards membrane-bound molecules (receptors of insulin and epidermal growth factor) [96, 99]. The increased content of sialoglycans in CD45 may cause masking of insulin receptors on organs and tissues, preventing the effect of minimal amounts of the hormone, which can still be produced in type 1 diabetes. This effect may disimprove complications during the development of the disease [96].

The α2,6-sialylation of leukocyte glycoconjugates undergoes certain changes in type 1 DM (**Figure 2**) [100]. Therefore, the quantity of sialic acids linked by α2,6-glycosidic bonds correlate with the disease duration. The content of sialoglycoconjugants on leukocytes surfaces increases for patients with the disease up to five years, while it decreases for patients with the disease duration over ten years. The pathology is accompanied by an increase of linkage places for SNA, which indicates the replacement of α2,3-linked sialic acids by α2,6-linked acids. It is likely to as a result of quantitative changes in the cells or in the enzyme activity of ST6Gal and/or ST6GalNAc [45]. The activity of α2,6 sialyltransferase decreases during the biosynthesis of O-glycans of T lymphocyte in the process of their activation. Thus, an increase in the content of α2,6-linked sialic acids of leukocyte cell surfaces along with a decline in the number of α2,3-linked sialic acids may indicate an increased sensibilization towards B lymphocyte stimulation and the inhibition of T lymphocyte activity under type 1 DM [20, 58].

6. Conclusions

Under cell–cell interaction, not only the presence of certain glycoconjugate, but also the type of linkage of sialic acids to the oligosaccharide chaine is informative. Against the general increase in the number of sialic acid-containing glycoconjugates on leukocytes surface under type 1 DM, there were small quantities of sialic acids linked by α2,3-glycosidic bond to subterminal carbohydrates in structure of glycans. Whereas, the quantity of sialic acid linked by α2,6-glycosidic bonds in the structure of sialoglycans correlated with the duration of diabetes. Such peculiarities of the structure of sialoglycoconjugates of leukocytes may affect both dynamic and kinetic indices of cell aggregation. Leukocytes aggregation affected by lectins may be used as a model of adhesion and migration of these cells. The abnormal redistribution of glycoconjugates on leukocytes membrane under type 1 DM causes changes in

their aggregation and adhesion to the vascular endothelium, as well as impairment of the phagocytic function of neutrophils. Thus, the accumulation of leukocyte aggregates in microvessels and violation of disaggregation mechanisms lead to damage of blood vessels. Such changes are etiological preconditions for the development of complications and chronic diseases resulting in deterioration in diabetics' conditions.

Conflict of interest

The authors declare no conflict of interest.

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