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РОЛЬ ГЛИКОДЕЛИНА В РЕГУЛЯЦИИ ИММУННОЙ СИСТЕМЫ В КОНТЕКСТЕ РАЗВИВАЮЩЕЙСЯ БЕРЕМЕННОСТИ

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Резюме. В обзоре представлены данные о роли гликоделина А (GdA, PP14, α2-PEG, EP15, PEP, AUP, PAEP) в регуляции функций иммунной системы в контексте формирования иммунной толерантности во время беременности.

Гликоделин был впервые выделен и идентифицирован в 1976 году Петруниным Д.Д. и Татариновым Ю.С. с коллегами как новый антиген плаценты, который был назван хорионическим α2-микроглобулином. С тех пор получено огромное количество научных данных о структуре, свойствах и биологических эффектах этого гликопротеина. Данный белок имеет четыре дифференциально гликозилированные изоформы, а именно GdA, GdF, GdC и GdS, которые секретируются в различных частях репродуктивного тракта.

Наиболее изученная изоформа, гликоделин А (GdA), секретируется децидуальным железистым эпителием и в процессе беременности накапливается в амниотической жидкости и материнской сыворотке. Уровень GdA служит признаком фертильной функции эндометрия. GdA обладает разнонаправленными биологическими эффектами, в частности модулирует эндокринную функцию и дифференцировку клеток трофобласта.

Роль GdA в регуляции иммунной системы заключается в ингибировании пролиферации Т- и В-лимфоцитов, подавлении цитотоксичности NK-клеток, индукции апоптоза активированных $CD4^+$ клеток, моноцитов и NK-клеток, угнетении активности цитотоксических Т-лимфоцитов и подавлении функциональной активности макрофагов и дендритных клеток. Помимо этого, GdA повышает уровень регуляторных Т-лимфоцитов, сдвигает баланс Th1/Th2 в сторону Th2 и индуцирует толерантный фенотип в дендритных клетках.

Иммуномодулирующая активность GdA зависит от степени его гликозилирования, которая, в свою очередь, связана со способом получения препарата. Поэтому в обзоре проанализированы особенности иммуномодулирующего действия нативного и рекомбинантного типов гликоделина на клетки иммунной системы.

Тем не менее суммарные эффекты GdA на клетки иммунной системы позволяют рассматривать его как один из основных факторов, формирующих иммунную толерантность организма матери к разви-

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вающемуся эмбриону. Важно также отметить, что клинические исследования выявили корреляцию между низким уровнем циркулирующего GdA с повторяющимися спонтанными абортами, что подтверждает важность этого белка в фетопротекции.

В целом, очевидно, что GdA имеет перспективы применения в биомедицине в качестве фармакологического препарата для лечения аутоиммунных заболеваний, посттрансплантационных осложнений и «перепрограммирования» аутореактивных клонов Т-лимфоцитов *in vitro* для дальнейшей клеточной иммунотерапии.

Ключевые слова: нативный гликоделин А, рекомбинантный гликоделин А, иммунная система, иммунная толерантность при беременности, иммуномодуляция

ROLE OF GLYCODELIN IN THE IMMUNE SYSTEM REGULATION IN THE CONTEXT OF DEVELOPING PREGNANCY

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Abstract. The review presents data on the role of glycodelin A (GdA, PP14, α2-PEG, EP15, PEP, AUP, PAEP) in regulation of immune system functions in the context of evolving feto-maternal immune tolerance during pregnancy. Glycodelin was first isolated and identified in 1976 by Petrunin D.D. and Tatarinov Yu.S. with colleagues as a new placental antigen, which was called chorionic α2-microglobulin. Since then, a huge amount of scientific data has been obtained on the structure, properties, and biological effects of this glycoprotein. This protein has four differentially glycosylated isoforms, namely GdA, GdF, GdC, and GdS, which are secreted in different compartments of reproductive system.

The most studied isoform, glycodelin A (GdA), is secreted by decidual glandular epithelium and accumulates in the amniotic fluid and maternal serum during pregnancy. GdA level is a marker of endometrial fertile function. GdA has diverse biological effects, in particular, as modulator of endocrine function and trophoblastic cell differentiation. The role of GdA in regulation of immune system is to inhibit T and B lymphocyte proliferation, suppress the NK cell cytotoxicity, induce apoptosis of activated $CD4^+$ cells, monocytes and NK cells, inhibit the cytotoxic T lymphocyte activity, and to suppress functional activities of macrophages and dendritic cells. In addition, GdA increases the levels of regulatory T cells, modifies the Th1/Th2 balance towards Th2, and induces a tolerant phenotype of dendritic cells.

The immunomodulating activity of GdA depends on the degree of its glycosylation, which, in turn, is associated with its preparation technique. Therefore, the review analyzed the features of the immunomodulating effects of the native and recombinant types of glycodelin upon the immune cells. However, cumulative effects of GdA upon the cells of the immune system make it possible to consider it among the main factors shaping feto-maternal immune tolerance during pregnancy. It is also worth of note, that clinical studies have revealed a correlation between low levels of circulating GdA, and repetitive spontaneous abortions that confirm importance of this protein for the fetal protection.

In general, it is obvious that GdA has a potential of medicinal application for treatment of autoimmune diseases, post-transplant complications and *in vitro* reprogramming of autoreactive T cell clones for subsequent cellular immunotherapy.

Keywords: native glycodelin A, recombinant glycodelin A, immune system, feto-maternal immune tolerance, immunomodulation

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General characteristics of glycodelin

Glycodelin is a human glycoprotein that belongs to the superfamily of lipocalins [69]. Members of this family are characterized by the ability to bind small hydrophobic molecules, cell surface receptors and soluble macromolecules [17]. Glycodelin was first isolated and identified by Petrunin D.D. and Tatarinov Yu.S. with colleagues in 1976 as a placenta new antigen, which was called chorionic α2-microglobulin [3]. With the accumulation of data on the localization and properties of the protein,

its name changed to placental α2-microglobulin, α2-microglobulin of fertility (AMGF) and, finally, specific α2-microglobulin. Later glycodelin was characterized by several independent research groups as placental protein 14 (PP14) [9], pregnancy-associated endometrial α2-globulin (α2-PEG) [6], endometrial protein 15 (EP15) [7], progestagen-dependent endometrial protein (PEP) [21, 23], alpha-2 uterine protein (AUP) [18, 74], progesterone associated endometrial protein (PAEP) [29]. To avoid terminological confusion, Dell A. et al. in 1995 [13] proposed a new protein's name: "glycodelin", reflecting its unique feature – sex-dependent glycosylation.

Structure of glycodelin

Glycodelin is a dimeric glycoprotein consisting of 180 amino acid residues (of which 18 are an N-terminal signal peptide), which molecular weight varies from 42 to 56 kDa, depending on the source and method of isolation [69]. Carbohydrates are about 20% of the molecular weight of the protein. The homodimeric form serves as the framework for four large side chains of sugar on the same surface of the protein. Different chains of oligosaccharides are responsible for a variety of biological functions in reproduction and immunosuppression [72].

The first studies of the N-terminal sequence of glycodelin revealed 59% identity with equine β-lactoglobulin and 23% identity with the human retinol-binding protein. The highest similarity of glycodelin – 91% is found with rhesus macaque. In β-lactoglobulins, four cysteine residues at positions 66, 106, 119 and 160 are responsible for intramolecular disulfide bridges, all of which are conserved in glycodelin [19, 25]. Despite these structural similarities, the amino acid sequence of β-lactoglobulin does not contain any glycosylation sites present in glycodelin [19, 31, 67, 69]. Notwithstanding the resemblance in the general folding patterns of glycodelin and β-lactoglobulin, the conformations of these proteins are different, which is determined by differences in their denaturation processes [31]. Also it was found that, unlike β-lactoglobulin, glycodelin A (GdA) does not bind to retinoids or any endogenous hydrophobic ligands [31, 35].

Comparative immunochemical studies, analysis of N-terminal sequences, and cDNA sequencing showed that the glycodelin isoforms are identical in the primary and tertiary structure of the protein molecule, in immunogenicity and in some physicochemical properties [67]. They differ only in glycosylation. Initially, three potential N-glycosylation sites were found at positions 28, 63, and 85, but later it turned out that only the first two of them were glycosylated [13, 40, 41, 45]. Mass spectrometric analysis of GdA and glycodelin S (GdS) N-glycans showed significant differences between them. The main differences in glycans among "female" isoforms of glycodelins, such as GdA, GdC and GdF are basically at the level of terminal sialic acid residues. GdA contains the most of all residues of sialic acids Galβ1-4GlcNAc (lacNAc), GalNAcβ1-4GlcNAc (lacdiNAc), NeuAcα2-6Galβ1-4GlcNAc (sialylated lacNAc), NeuAcα2-6GalNAcβ1-4GlcNAc (sialylated lacdiNAc), Galβ1-4 (Fucα1-3), GlcNAc (blood group Lewisx) and GalNAcβ1- (Fucβ1-3) GlcNAc (analogue of lacdiNAc Lewisx), opposite to glycodelins C (GdC) least. Glycan profile of glycodelin F (GdF) is similar to GdA profile, except that GdF contains less sialic acid residues. GdS does not contain sialylated glycans, its glycans are extraordinarily rich in fucose, and the basic structures of a complex type are two antennae glycans with Lewisx and Lewisy [Fucα1-2Galβ1-4 (Fucα1-3) GlcNAc] [45]. GdA and GdS have identical primary structures, immunoreactivity, tryptic peptide profiles, and similar thermodynamic parameters of reversible denaturation [30, 31], but different glycosylation [35]. Oligosaccharides containing terminal sialylated lacNAc or lacdiNAc residues present in GdF and GdA may exhibit immunosuppressive effects, whereas less sialylated GdC and unsialylated GdS do not possess significant immunosuppressive activity [45, 47]. However, the ligand for glycodelin has not yet been identified – there are only assumptions in the literature that, depending on glycosylation rate, glycodelin involve in their effects realization a number of molecules – L-selectin, E-selectin, CD45, CD22 and SIGLEC-7, as will be discussed below.

Place of glycodelin synthesis

The protein has four differentially glycosylated isoforms, namely GdA, GdF, GdC and GdS, which are secreted in various parts of the reproductive tract [68, 84]. Because of differences in glycosylation, the isoform of glycodelin isolated from the amniotic fluid was called GdA, and the corresponding glycodelin isoform isolated from seminal plasma was called GdS. Immunomorphological and immunochemical studies showed that GdA appears in the endometrial tissues a few days before the implantation, its amount increases during the implantation window and remains high until the onset of menstruation and during the first days of the next cycle, after which expression of the protein in the endometrium temporarily stops. In the case of pregnancy, GdA synthesis continues, and its content in decidual tissue in the first trimester reaches 4-10% of the total protein [69]. Thus, GdA is secreted mainly by decidual glandular epithelium and in the case of pregnancy, the protein accumulates in the amniotic fluid and maternal serum [21, 22]. The concentration of GdA in maternal serum at 12 weeks is 1200 μg/l, and at 40 weeks – 100 μg/l, and in the amniotic fluid the concentration of protein at 12 weeks is 13 mg/l, at 16 weeks – 125 mg/l, and at 40 weeks – 1 mg/l [24]. During a typical menstrual

cycle, GdA reaches serum concentrations in the middle of the proliferative phase – < 20 μg/l, in the middle of the luteal phase – 35 μg/l, in the late luteal phase – 47 μg/l, during menstruation – 74 μg/l [27]. GdF is secreted in the ovarian follicles [78], and GdC in multilayered epithelium cells in the wall of the vesicular ovarian follicle (*cumulus oophorus*) [10]. GdS is the only isoform found in the male body in seminal plasma [26, 84], whereas other isoforms are found only in women. Glycodelin has also been identified outside the reproductive system in cells of the megakaryocyte lineage, including platelets [46]. Other authors have shown that GdA (PAEP) is expressed in the hematopoietic system by erythroid progenitors, but not by mature erythrocytes, platelets, mononuclear phagocytes or lymphocytes [29]. Finally, it is worth noting that GdA expression is observed not only in healthy tissues of the reproductive tract, but also in certain types of cancer: lung, breast, endometrium, ovaries, and melanoma cells [28, 57, 64, 65, 82]. It has been shown that GdA can be used as a biomarker to track the progression of a tumor, as has been shown in non-small cell lung cancer, recurrence or metastatic spread [11]. Understanding the regulation of the glycodelin expression may lead to the development of new therapeutic approaches, with the help of which it will be possible to weaken the system of protection of the tumors themselves.

Clinical and diagnostic value of glycodelin

In clinical studies, glycodelin is a prognostic marker of early fetal loss and male infertility [2, 12]. GdS is a sensitive marker of fertility and its definition can be used as an additional test for the early detection of the pathology of ejaculate in idiopathic infertility [1]. The level of GdA is a sign of endometrial fertility. Serum GdA concentration is an important parameter for monitoring the menstrual cycle [8]. It can be used to distinguish the ovulatory menstrual cycle from non-ovulatory, which provides valuable information for the diagnosis of infertility *in vitro*. Additionally, this test is used to determine the optimal embryo transfer time in *in vitro* fertilization protocols [75] and in the diagnosis of the threat of termination of pregnancy [76]. Reducing expression of glycodelin can cause activation of the maternal immune system, and ultimately lead to the rejection of the developing embryo [77].

Biological effects of GdA

GdA is the most widely studied and most interesting isoform from all glycodelins. It is positioned as a specific protein of the human reproductive system. GdA inhibits the binding of spermatozoa to *zona pellucida* and protects spermatozoa from an immune attack in the maternal reproductive tract [85]. GdA promotes successful implantation and subsequent development of pregnancy [75]. Thus, a decrease in its level is associated with an increased risk of preeclampsia and recurrent abortions [34, 36]. Simultaneous expression of enzymes that destroy the matrix and their inhibitors in trophoblasts suggests that the balance between these components regulates the invasive activity of trophoblast cells. GdA is one of the decidual factors that limit the invasion of trophoblast [34]. It is important to note that this effect is strictly dependent on the glycosylation of the molecule since deglycosylation and even minor changes in glycosylation present in various glycoforms decrease this activity [34]. In addition, GdA regulates the development of the placenta, which is closely related to the processes of angiogenesis. The angiogenic effect of GdA is mediated by the enhancement of vascular endothelial growth factor (VEGF), which is involved in placental angiogenesis [40]. Since glycodelin has been found in the glandular structures of many tissues, including seminal vesicles, lobular and ductar epithelium of the mammary gland, eccrine sweat glands and parabronchial glands, it is suggested that it plays the role of a marker of differentiation and morphogenesis in glandular tissues [69]. GdA is able to induce trophoblast cells to produce chorionic gonadotropin, an extremely important hormone that accompanies the development of pregnancy [20]. The authors conclude that *in vivo* GdA modulates the endocrine function, as well as the differentiation of trophoblasts. Thus, GdA has multiple biological functions.

Immunomodulatory effects of GdA

Obviously, immunocompetent cells of the reproductive tract fall under the influence of GdA. It is known that in the early stages of pregnancy in the decidua, about 40% of the stromal cells are leukocytes, of which 45-70% are NK cells, 30% are macrophages and less than 20-30% are $CD3^+$T cells. During pregnancy, $CD3^+$ cells frequency remains stable, but in the third trimester their number increases, and the number of NK cells decreases [61].

Cells of the monocyte-macrophage lineage

Macrophages are also found in large numbers in the decidua. Their quantity is regulated by the hormones of the ovaries since macrophages contain receptors for estrogens. One of the key functions of these cells is the timely elimination of apoptotic cells [4]. With successful elimination, the production of Th2-cytokines by decidual macrophages increases, whereas the inferior removal of apoptotic cells leads to the hyperactivation of macrophages and the enhancement of pro-inflammatory Th1-cytokines production by them. Decidual macrophages help to maintain immune tolerance towards fetal antigens and to protect fetus from the constant risk of infection [42].

In order to analyze the effects of glycodelin on the cells of the immune system, it is important to separate the two available approaches: the use of native glycodelin derived from amniotic fluid or homogenate

of decidual tissues and the use of recombinant protein. Both the first and second approaches have their methodological differences associated with the degree of purification of the preparation, the source of the recombinant protein, the presence of isoforms, and the degree of glycosylation. Nevertheless, our task is to generalize the available information and to try to understand the dominant vector of the effects of this complex protein.

Thus, it is known that GdA dose-dependently inhibits the ability of human monocytes (cell line U937) to chemotaxis, losing these properties after deglycosylation [79].

Miller R.E. and colleagues found that human recombinant GdRec protein isolated from *E. coli* BL21 (DE3) periplasm transformed with the bacterial expression vector pPP14.1 EE his6/ET-22b ($^+$) specifically bound to $CD14^+$ cells (monocytic cell line), but not with $CD20^+$ cells (B cell line) and $CD3^+$ cells (T cell line). Thus, the authors show the presence of a receptor for this protein on the membrane of the $CD14^+$ cells of the monocytic cell line [43].

Neither glycosylation nor sialylation of glycodelin does not impair the ability of monocytes to chemotaxis. Recombinant GdA binds to a specific protein receptor in monocytes, but the corresponding receptor is not detected on the surface of T or B cells [11]. Further study of the GdA action mechanism established that on the surface of cells of the monocyte-macrophage lineage, the binding protein for glycodelin is the L-selectin molecule. Thus, treatment with antibodies against L-selectin reduced the binding of GdA and the GdA-induced production of IL-6 [39].

When comparing the effects of native GdA derived from an amniotic fluid and recombinant glycodelin (GdRec), both of these proteins have been shown to inhibit both T cell and monocytic cell lines (U937 [48] and THP1 [5]) proliferation. A more detailed study of the GdRec effects has shown that it induces apoptosis of THP1 cells, as well as apoptosis of monocytes obtained from healthy volunteers. When studying the mechanism of action, it was demonstrated that GdRec realizes its apoptotic effect suppressing the expression of the anti-apoptotic genes Bcl-2A1 and APRIL and increasing the expression of the proapoptotic genes TNF-R1, Bad and Bax. At the same time, GdRec had no effect on phagocytosis processes in THP1 cells [5].

In 2012, Lee and colleagues demonstrated that GdA, which was obtained from amniotic fluid by affinity chromatography, does not affect the viability, cell death, and monocyte /macrophage phagocytosis, but induces the secretion of IL-6 by these cells [39]. At the same time, non-glycosylated GdRec did not have a stimulating effect on IL-6 production. GdA-induced IL-6, in turn, inhibited the expression of IFNγ by T lymphocytes by an autocrine mechanism, which led to the Th2 activation. The anti-inflammatory effect of GdA is supported by the fact that it suppresses the production of TNFα by macrophages obtained from monocytes without affecting their phagocytic activity [39].

In a recent paper, Vijayan and colleagues discovered a possible GdA receptor on the human peripheral blood monocytes membrane. The GdA receptor – SIGLEC-7 (Immunoglobulin-like lectin 7 binding sialic acid; CD328) was identified by co-immunoprecipitation and flow cytometry. In culture conditions, GdA enhanced the expression of IDO-1 and CD209 decidual macrophage markers on GdA-polarized macrophages. Blocking the SIGLEC-7 receptor on cells leveled the biological effects of GdA on monocyte differentiation [80].

Dendritic cells (DC) are present in the maternal part of the placenta and are represented by immature and mature myeloid dendritic cells, with the cells responsible for the induction of T-cell anergy. Placental DC and macrophages actively absorb extravillous trophoblast cells undergoing apoptosis, which is considered as a stage of induction of the mother's immune tolerance to fetal antigens inherited from the father. Thus, it is known that a native GdA preparation derived from an amniotic fluid induces a tolerogenic phenotype of DCs (IL-10-producing cells) derived from monocytes *in vitro* [66]. At the same time, GdA suppressed maturation of DCs from peripheral blood mononuclear cells: after preliminary treatment with GdA, immature DCs could not go into a full mature phenotype. GdA suppressed the expression of costimulatory molecules CD83 and CD86 on immature DCs. Co-cultivation of obtained in this way DCs with allogeneic mononuclear blood cells dose-dependently reduced their lymphoproliferative activity [11]. Glycodelin isolated from ascites of patients with ovarian cancer, also prevents the maturation of DCs with a tolerogenic phenotype and functionality, suggesting that glycodelin can form an immunosuppressive microenvironment in the progression of cancer [11].

NK cells

At the beginning of pregnancy, the dominant cells in the decidua are uterine NK cells, which constitute up to 80% of all leukocyte infiltrate cells. NK cells lose their CD16 (FcRγIIIA), which provides the ability to carry out a cytotoxic effect, and start to express on the membrane HLA-G (human leukocyte antigen-G) tolerogenic molecule, through which they interact with the trophoblast, modulate immune processes in the endometrium, and also produce angiogenic factors [16]. Interestingly, glycodelin is selectively expressed in decidual NK cells and is practically not identified in peripheral NK cells [32]. As early as in 1991, it was demonstrated that glycodelin, obtained

from amniotic fluid, blocks the cytotoxicity of NK cells against the K562 cell line [50]. The authors suggest that GdA is thus involved in preventing fetus immune rejection in the fetoplacental interface.

Lee C.L. with colleagues studied the effects of GdA, isolated from amniotic fluid, on the functional activity of peripheral NK cells. It has been shown that glycodelin high concentration (1μg/mL) significantly reduced the cytotoxicity of NK cells against target cells, although the ability of binding NK cells to target cells is preserved [37]. GdA did not affect the viability, cytotoxicity, and phenotype of peripheral blood NK cells, but increased the secretion of IL-6, IL-13, and GM-CSF by them [37].

B cells

The B-cell content in the decidua is small (as in the mother's bloodstream), but it increases significantly in the course of pregnancy, reaching 13% in last trimester. Th2 bias during pregnancy facilitates the development of the humoral immune response, including in the fetomaternal interface. Yaniv and colleagues showed that GdRec (recombinant form of GdA, PP14 • Fcγ1) regulates humoral immunity, suppresses proliferation, IgM secretion and MHC class II expression on stimulated B cells, without affecting other surface molecules such as CD69 and CD86 [83]. In the experiments of Alok and co-authors, it was demonstrated that *S. aureus*-activated B cells under the influence of GdA reduced proliferative activity, evaluated in the H3-thymidine incorporation assay [5]. In addition, the proliferation of the human B-cell line U266B1 also decreased under the influence of GdA [5]. Quite interestingly, similar effects in this study were obtained both with the use of native GdA derived from amniotic fluid and recombinant GdA obtained in insect cells (Sf21, ie, *Spodoptera frugiperda* or Mb, ie, *Mamestra brassica* [5]). It is known that oligosaccharide chains in native glycodelin specifically interact with CD22 on B cells and have immunosuppressive functions [11, 13].

T cells

It is known that the development of physiological pregnancy is associated with the formation of immunological tolerance to the alloantigens of the paternal haplotype, which are expressed by the embryo. In general, the current concept of immunological tolerance is that during a normal pregnancy, changes occur in the mother's immune system, such as the dominance of Th2 and regulatory T cells (Treg) over Th1 and IL-17-producing T cells (Th17), as well as an increase of the indoleamine-2,3-dioxygenase (IDO) level [59].

It is known that native GdA can inhibit T-cell proliferation in a mixed culture, suppressing the mitogenic response of lymphocytes to phytohemagglutinin (PHA) [53]. It has been shown that inhibition of activation and proliferation of T cells by the native preparation of GdA is carried out by inhibiting the transmission of T-cell receptor signals [55, 56].

As for $CD8^+$T cells, the native GdA preparation, obtained by immunoaffinity chromatography from the amniotic fluid, reduces the cytotoxic effects of alloactivated $CD8^+$T cells. Inhibition of cytotoxic T-lymphocytes activity is caused by suppression of transcription of effector molecules (granzyme B and perforin), and blocking the degranulation of cytolytic vesicles. Despite the inhibition of $CD8^+$T-cells proliferation, GdA does not affect the initiation of apoptosis in these cells [70].

When studying the mechanism of GdA action, it was found that it participates in early signal transduction, involving T cell receptor (TCR, CD3). First, it abolishes the TCR-induced flow of Ca^{2+} ions in early TCR-CD3 signaling events. Unlike other T cell inhibitors, such as cyclosporin A, that inhibit Ca^{2+}-dependent phosphatase (calcineurin) to weaken the signaling pathway of activation, regardless of interaction with TCR, GdA, on the contrary, increases the TCR activation threshold and changes the corresponding profile of cytokine expression, but not directly blocks the transduction of the T-cell signal. In addition, GdA can be localized in APC – T cell contact sites at TCR startup to inhibit activation of T lymphocytes. In addition, the binding of GdA to intact CD45 molecule of T cells diminishes the TCR-CD3 signal transduction [11].

In 2012, it was found that GdA disrupted IL-2/IL-2R signaling processes in T cells by reducing the expression of IL-2R on the cell surface, which resulted in a T cell proliferation decrease. It has generally impaired the immune response, including by weakening the cytotoxicity of $CD8^+$T cells. Given that IL-2 regulates the expression of pro- and anti-apoptotic proteins in activated cells, an insufficient IL-2-signal in the presence of GdA may cause a decrease in the anti-apoptotic Bcl-2 protein and an increase in the pro-apoptotic Bax protein, which contribute to the induction of $CD4^+$T cells apoptosis [71]. GdA also causes mitochondrial stress, which directly leads to apoptosis of T cells independent of TCR and CD45 signals [73]. GdA can induce apoptosis in activated T cells by selectively combining N-linked glycans on the glycoproteins of the T cell surface since activated T cells express more galactose than naïve T cells [48]. It is important to note that all these conclusions are drawn from the analysis of the effects of the native GdA preparation, obtained by the immunoaffinity chromatography from the amniotic fluid. In addition, GdA-mediated induction of apoptosis in activated T cells occurs through permeabilization of the mitochondrial membrane and induction of caspase-3, caspase-9 [38, 48, 73], increasing the activity of caspase-8, which leads to increased expression of

Fas, and thereby increase cell mortality [38]. Both recombinant glycodelin GdRec obtained from *P. pastoris* cells (GS115 strain) which does not contain sialic acid residues and the desialylated form of native GdA do not induce T-cell apoptosis. The authors conclude that the apoptotic activity of GdA depends on the presence of sialic acid residues in this protein [48].

GdA is involved in the regulation of the functional activity of the dominant regulatory subpopulations of helper T cells – Th1 and Th2. For example, the recombinant form of GdA (PP14 • Fcγ1) inhibits the polarization of naïve $CD4^+$T cells towards the Th1 during T cell priming, suppressing the expression of IFNγ, IL-2 and CXCR3 (chemokine receptor), which are important for the development and activation of Th1 cells [44]. At the same time, native GdA induces apoptosis of Th1 cells mainly by enhancing Fas expression in them in comparison with Th2 cells [38]. GdA-treated macrophages exerted a suppressive effect on the intracellular level of IFNγ in helper T cells. With a detailed study of the native GdA action mechanism, it has been shown that it realizes its Th1-suppressive effect through the induction of IL-6 production by macrophages. At the same time, IL-6 promotes the differentiation of Th-2 cells, and GdA, thus, can improve fetal survival and help maintain pregnancy [39]. Thus, the general vector of the effects of recombinant and native GdA is directed to the formation of Th2-bias of immune response necessary for the normal pregnancy. In the experiments of Pockley et al., it was shown that first trimester decidual tissue extracts in which GdA is present inhibit the production of IL-2 in PHA-stimulated lymphocytes and lead to a reduced release of the IL-2R receptor [52]. The same authors showed that first trimester decidual tissue extracts and the purified GdA preparation suppressed the production of IL-1 in mitogen-stimulated (PHA and LPS) cultures of mononuclear cells [53].

However, in addition to the dominant Th1/Th2 subpopulations, generation of antigen-specific clones of regulatory T cells (Treg) is of great importance during pregnancy [59]. Treg accounts for 5-10% of the total population of helper T cells. They support immunological tolerance, participate in the suppression of the final stages of immune response, prevent the development of autoimmune diseases and grafts rejection. Treg enter the endometrium from the peripheral blood, the maximum of this cell's number in the decidua is observed in the first trimester of pregnancy, and Treg elimination leads to its interruption [33].

It is known that GdRec, obtained from the cell line of human embryonic kidneys, that has the same type of carbohydrate structures as amniotic (HEK 293 – Human Embryonic Kidney 293), under prolonged culture conditions increased the level of myelin-specific Treg and the expression of FoxP3 *de novo* with simultaneous suppression of effector T cells. In the experiment, there was a twofold increase in the number of $FoxP3^+$T cells, which were also characterized as $CD25^{high}$ and $GITR^{high}$ [49]. The observed increase in FOXP3 expression in cells treated with GdA was the result of premature termination of signaling via TCR and in particular the PI3K/Akt/mTOR pathway. By regulating the mTOR signaling, glycodelin can influence the antigen-induced differentiation of T effectors or Treg [49]. The authors conclude that GdA has a potential therapeutic effect on T cells in autoimmune diseases by preventing the development of effector T cells and inducing antigen-specific Treg. In the long term, GdA would be logical to use in cellular immunotherapy for "reprogramming" autoreactive clones of T cells and directed induction of necessary antigen-specific Treg.

It should also be noted that physiological pregnancy is accompanied by a decrease in the frequency of proinflammatory IL-17-producing helper T cells (Th17) in peripheral blood compared with non-pregnant women [60]. Elevation of the Th17 number, in turn, is associated with pathological processes and can lead to premature birth or spontaneous abortion [59]. GdA-treated macrophages, when co-cultured with autologous lymphocytes, did not have a significant effect on the intracellular IL-17 level of T helper cells [39].

In 2018, the role of the immunosuppressive activity of the recombinant GdA (source of *E.coli* C43) in preventing graft rejection was investigated [14]. Using an *in vitro* experimental model based on the co-cultivation of mononuclear cells with targeted HepG2e cells, it was demonstrated that GdA suppressed the generation of cytotoxic lymphocytes and their functional activity. Similar results were obtained *in vivo* in nude mice that became recipients of HepG2e cells and GdA-treated human $CD8^+$ cells. It was shown that *in vivo* GdA also inhibits the activity of cytotoxic lymphocytes [14].

In the same 2018, Paloma Riquelme and colleagues received regulatory macrophages (Mregs) from human blood monocytes $CD14^+$ *in vitro*. By co-cultivation of Mregs with $FoxP3^-CD4^+$T cells, the researchers demonstrated that Mregs promoted the formation of IL-10-producing $FoxP3^+$ (Treg) regulatory T cells, the so-called Mreg-induced (miTreg) cells, which subsequently suppressed nearby T-cells and prevented maturation of dendritic cells in culture. When anti-GdA antibodies were added to the mixed Mreg/$FoxP3^-CD4^+$T cells culture, a significant decrease in miTreg generation was observed. The authors concluded that under co-culture conditions,

TABLE 1. EFFECTS OF DIFFERENT TYPES OF GLYCODELIN A ON THE FUNCTIONS OF IMMUNE CELLS (IN CHRONOLOGICAL ORDER OF DISCOVERY)

Type of glycodelin	Effects	Reference
Purified extract of decidual tissue, I trimester	Reduction of proliferative activity of lymphocyte mixed culture and PHA-stimulated lymphocytes	Bolton et al., 1987
Purified extract of decidual tissue, I trimester	Reduction of production of IL-1 and IL-2, as well as sIL-2R by mitogen-stimulated lymphocytes and mononuclear cells	Pockley et al., 1988
Amniotic fluid, second trimester	Inhibition of cytotoxicity of NK cells against the K562 cell line	Okamoto et al., 1991
Recombinant GdA (from E. coli BL21 (DE3), transformed with bacterial expression vector pPP14.1 EE his6/ ET-22b (+))	Specifically associated with $CD14^+$ cells (monocytic cell line), but not with $CD20^+$ cells (B cell line) and $CD3^+$ cells (T cell line)	Miller et al., 1998
Native* and recombinant (PP14 Fcγ1) GdA	Reduced T cell activity (proliferative)	Rachmilewitz et al., 1999, 2001
Native* and recombinant	The inhibitory effect on the T cell proliferation is mediated through binding to α2-macroglobulin	Riely et al., 2000
Native and recombinant	Directly induces apoptosis of T cells, regardless of monocytes	Mukhopadhyay et al., 2001
Homogenate of decidual tissue	Decreased chemotaxis of U937 monocytic cell line	Vigne et al., 2001
Recombinant form, PP14•Fcγ1	Suppress proliferation, IgM secretion and MHC class II expression by stimulated B cells	Yaniv et al., 2003
Recombinant form of GdA (PP14•Fcγ1)	Prevents polarization of naïve $CD4^+$ T cells towards Th1, suppressing the expression of IFNγ, IL-2 and CXCR3	Mishan-Eisenberg et al., 2004
Native GdA	Induced mitochondrial stress, which directly leads to apoptosis of T cells independent of TCR and CD45 signals	Sundarraj et al., 2008
Native GdA	Reduced proliferation of *S. aureus*-activated B cells and proliferation of U266B1 B cell line	Alok et al., 2009
Native GdA	No effect on viability, peripheral NK cell phenotype, but increased secretion of IL-6, IL-13 and GM-CSF	Lee et al., 2010
Native GdA	Reduced cytotoxicity of allo-activated $CD8^+$T cells	Soni et al., 2010
Recombinant GdA derived from Human Embryonic Kidney 293 cell line (HEK 293)	*In vitro* increased the level of antigen-specific Treg and the expression of FoxP3 *de novo* with simultaneous suppression of effector T cell functions	Ochanuna et al., 2010
Native GdA	Induced apoptosis of Th1 cells mainly by enhancing the expression of Fas in them in comparison with Th2 cells	Lee et al., 2011
Native GdA	It did not affect the intracellular level of IL-17 expression in T-helper cells when co-cultured with GdA-treated macrophages	Lee et al., 2012

Type of glycodelin	Effects	Reference
Native GdA	Violated the IL-2/IL-2R signaling processes in T cells, which led to a decrease in cell proliferation	Soni et al., 2012
Recombinant GdA (*E. coli* source C43)	Prevented nude mice transplant rejection	Dixit et al., 2018
GdA secreted by human regulatory macrophages *in vitro*	Under co-culture (Mregs and FoxP3$^-$CD4$^+$T cells), GdA (PAEP) secretion by Mreg promotes Treg induction	Riquelme P. et al., 2018

Note. *, native GdA preparation obtained by immunoaffinity chromatography from amniotic fluid.

Mreg secrete GdA (PAEP) and thus induce miTreg generation from FoxP3$^-$CD4$^+$T cells. The preoperative administration of Mregs of kidney donor to recipients led to a dramatic increase in circulating Tregs and promoted the transplant engraftment [58].

In studying the molecular mechanisms by which GdA suppresses transplant rejection, it has been shown that the protein reduces the number of activated CD4$^+$ and CD8$^+$ cells and reduces the expression of key proteins involved in graft rejection such as IL-2, granzyme-B, eomesodermin (EOMES), and production of pro-inflammatory cytokines (TNFα and IL-6), which leads to a weakened cell-mediated immune response. In addition, GdA induced apoptosis in CD4$^+$T cells, which are key mediators of the immune response. As a result, the authors see the possibility of using glycodelin in the therapy of rejection of the transplant. A similar assumption about the possible use of GdA in the case of lung transplantation was made by Schneider et al. [62, 63]. It is also important to note that clinical studies have revealed a correlation between a low level of circulating GdA with repeated spontaneous abortions, which confirms the importance of this protein in fetoprotection [12].

In general, it is obvious that GdA has prospects for use in biomedicine as a pharmacological drug for the treatment of post-transplant complications [14, 58] and autoimmune conditions [51] and "reprogramming" of autoreactive clones of T lymphocytes *in vitro* for further cellular immunotherapy.

Conclusion

Glycodelin (PP14, PAEP, alpha-2-microglobulin, a dimeric glycoprotein with a molecular weight of 42 to 56 kDa) is considered a marker of reproductive tissue receptivity [81]. In the normal course of pregnancy, the glycodelin level gradually increases, reaching a maximum at the 4-16th week of pregnancy, then begins to decrease, forming a plateau after 24 weeks [22, 24]. Clinical studies have revealed a correlation between a low level of circulating glycodelin with repeated spontaneous abortions [12], and with the development of preeclampsia [15], which confirms the importance of this protein in fetoprotection.

As a result, the data of modern literature show that the role of GdA in the regulation of the immune system is to inhibit the proliferation of T and B lymphocytes, suppress the cytotoxicity of NK cells, induce apoptosis of activated CD4$^+$ cells, monocytes and NK cells, inhibit the activity of cytotoxic T-lymphocytes and suppression of the functional activity of macrophages and dendritic cells (Table 1). In addition, GdA increases the level of Treg, bias the Th1/Th2 balance towards Th2 and induces a tolerant phenotype of dendritic cells. Immunomodulating activity of GdA depends on the degree of its glycosylation. The overall effects of GdA at the level of the immune system allow us to consider it as one of the main factors that form the fetomaternal immune tolerance, along with other pregnancy proteins (chorionic gonadotropin, pregnancy specific β1-glycoproteins, alpha-feto-protein, etc.).

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