Lectin-based analysis of fucose and sialic acid expressions on human amniotic IgA during normal pregnancy

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Abstract The sugar moiety of IgA is known to provide a link between the innate and adaptive immune systems. Terminally located glycotopes on IgA are potential ligands engaged in the interactions which may modulate the biological activities of IgA. In the present work the expressions of *Maackia amurensis* (MAA), *Sambucus nigra* (SNA), *Lens culinaris* (LCA), *Tetragonolobus purpureus* (LTA), and *Ulex europaeus* (UEA) reactive glycotopes on maternal plasma and amniotic IgA were evaluated in relation to the progression of a normal human pregnancy, from the 2nd trimester, throughout the 3rd trimester, perinatal period, post-date pregnancy and delivery, by lectin-IgA-ELISA, using specific biotinylated lectins. The amniotic and maternal plasma IgA concentrations and a degree of SNA and LCA reactivity of maternal plasma IgA were almost unaltered during the normal pregnancy. The amniotic IgA from the 2nd trimester was decorated by MAA-, SNA-reactive and LCA-, LTA-, and UEA-reactive glycotopes. At the turn of the 2nd and 3rd trimesters the expression of MAA-, SNA-, LTA-, and UEA-reactive glycotopes, except for LCA-reactive, increased and remained almost at unaltered levels throughout the perinatal period and delivery. However, in the post-date pregnancy the expression of LCA-, LTA-, and UEA-reactive and SNA-reactive glycotopes were significantly higher. The unique fucosylated and sialylated glycovariants of amniotic IgA associated with the progression of the normal pregnancy may illustrate a general importance of carbohydrate-lectin receptor interactions in the control and modulation of biological events to ensuring homeostasis during pregnancy, protection and well-being of fetus.

Keywords Amniotic fluid · Fucosylation · Glycovariant · Glycotope · Immunoglobulin A · Lectin · Pregnancy · Sialylation

Abbreviations

IgA	Immunoglobulin A
LCA	*Lens culinaris* lectin
LTA	*Tetragonolobus purpureus* lectin
MAA	*Maackia amurensis* lectin
SNA	*Sambucus nigra* lectin
UEA	*Ulex europaeus* lectin
Fuc	Fucose
SA	Sialic acid

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Introduction

In the biological development processes the defined oligosaccharide structures appear in specific spatial and temporal patterns [1]. During pregnancy, when intensive growth and development of the fetus occur, glycans terminated with sialic acid and/or fucose are known to control and modulate biological reactions involved in cell-cell signaling, adhesion, and stimulate cell growth and differentiation [2–4]. Additionally, the glycoconjugates bearing sialyl-Lewis antigens participate in the prevention of leucocyte adhesion to the fetal syncytiotrophoblast [5], and can interact with

amniotic selectins and play an immunomodulatory role in the late pregnancy [4, 6].

The glycosylation process during the pregnancy is stimulated and controlled by the endocrine changes [7], different sets and ratios of glycosyltransferases produced and activated in many clones of B-cells [8], as well as cytokines (IL-6) [7, 9]. Some glycoproteins of amniotic fluid, such as glycodelin, transferrin [10], human chorionic gonadotropin [11], α1-acid glycoprotein [12], fibronectin [13] and IgG [14], represent different schemes of glycosylation, particulary fucosylation and sialylation, in comparison with the glycoproteins of the maternal serum [4]. Moreover, glycosylation patterns of amniotic glycoproteins are reported to change throughout gestation because of different sites of biosynthesis (maternal, fetal, and placental) at subsequent stages of pregnancy [4, 15, 16].

In human amniotic fluid IgA is much less abundant than IgG. It can originate from maternal, fetal and placental tissues. The share of IgA in amniotic fluid derived from different sites of synthesis (local production in the decidua as well as by fetal mucosa), and forms of its transport (a nonspecific diffusion and receptor-mediated transport of maternal IgA) is known to vary with gestational age [17, 18]. The maternal IgA, like IgG, is potentially available to the embryo as early as on the 6th week of gestation [19]. The placental transfer of IgA starts when the first placental villi are formed and increases as gestation advances [19]. In contrast to IgG, amniotic IgA of fetal origin is more in favor of a preimmune role by natural polyreactive IgA antibodies similar to that reported for secretion [20]. Moreover, IgA in amniotic fluid is involved in the protection of the fetus against maternal IgG autoantibodies [21]. According to Quan et al. [18] the amniotic immunoglobulins, both IgG and IgA, can serve as a protection against pathogens and act through mechanisms similar to those observed in the female genital fluid. The amniotic pool of IgA, similarly to the serum IgA [22], consists mainly of monomeric form with a low level of secretory IgA (SIgA), and with various amounts of free secretory component (SC) [18]. Additionally, an unusual form of fetal immunoglobulins, absent in the maternal serum, was observed. This molecule contains no α chain but includes a Fabγ fragment non-covalently associated with SC and can act as an immune barrier against the infection and against autoantibodies derived from the mother [18].

Immunoglobulins A, besides their high molecular heterogeneity related to the fact that they can exist as monomers, dimers and oligomers, and additionally in IgA1 and IgA2 subclasses, and as a SIgA, occur in diverse glycoforms with the attached N- and O-glycans. The sugar part of IgA depends on the origin, meaning the systemic and mucosal immune systems [23, 24], and ranges from 6 % for serum IgA up to 25 % for secretory component (SC) [22, 25, 26]. IgA1, IgA2 and SIgA may carry distinct populations of N- and/or O-glycan structures, in part as the result of different glycosylation machinery for epithelial and plasma cells [22, 25–27]. Royle et al. [25] have reported that a secretory component of colostrum SIgA contains α1,4-, α1,3-, and α1,2- fucosylated and α2,3-sialylated glycans that are decoys for pathogens binding to mucosal surfaces. The glycosylation pattern of amniotic IgA during the pregnancy has not yet been elucidated and thus is of major interest since it is associated with different origins and functions.

In our study, we analyze the alternations in the expression of MAA- and SNA-reactive glycotopes and LCA-, LTA-, and UEA-reactive glycotopes of IgA in amniotic fluid during the normal human pregnancy from the 2nd trimester, throughout the 3rd trimester, perinatal period, post-date pregnancy and delivery. The levels of sialylation and fucosylation of the IgA were determined by lectin-IgA-ELISA using lectins with known specificity toward different types of sialic acid and fucose linkages Table 1 [28–32]. The sialylation and fucosylation patterns were determined on plasma and amniotic IgA, isolated by modified polyclonal anti-α chain antibodies. It should be pointed out that this approach did not determine the 'true' structure of the human IgA glycans, but allowed to observe the changes of glycotope expressions accessible to the interactions with lectins. Such glycan-lectin interactions between the lectin-receptors and the sialyl- and fucosyl-glycotopes are emerging as key elements in a wide range of pathophysiological processes.

Materials and methods

Patients and sampling

Samples of amniotic fluid (101) and blood plasma (101) were obtained from pregnant women (21–38 years old) with

Table 1 The major binding specificities of used lectins

Origin and used abbreviation of lectin	Binding preference
Maackia amurensis (MAA)	Terminal sialic acid linked α2-3 to Gal of N- and O-glycans [28]
Sambucus nigra (SNA)	Terminal sialic acid linked α2-6 to Gal/GalNAc of N- and O-glycans [29]
Lens culinaris (LCA)	Fucose linked α1-6 to proximal GlcNAc of the trimannosyl core of biantennary N-glycans [30]
Tetragonolobus purpureus (LTA)	Fucose linked α1-3 to GlcNAc (in Lewis^x) of N- and O-glycans [31]
Ulex europaeus (UEA)	Fucose linked α1,2 to Gal < Fucα1-2Galβ1, <Fucα1-3 or 1-4GlcNAc (in Lewis^x or Lewis^y) of N- and O-glycans [32]

gestational age between 14 and 42 weeks, receiving prenatal care at the Department of Obstetrics and Gynaecology, Wrocław Medical University, Wrocław, Poland. During the 2nd and 3rd trimesters, the amniotic fluid was taken by transabdominal amniocentesis under ultrasonographic guidance, and during delivery by transvaginal amniotomy. The samples used for the current study consisted of the fluid remaining after the performance of the routine diagnostic procedures. At 14–18 weeks of gestation, amniotic fluid was obtained by amniocentesis for fetal karyotyping. In all cases, an accurate gestational dating was established by obtaining knowledge about the most recent menstrual period and confirmed by the ultrasonographic evaluation. There were no signs of inflammation, vaginal infection, or chorioamnionitis. All samples were collected after the informed consent of the individual women, and the study was approved by the local ethics committee (no. KB-479/2006). Immediately after amniocentesis, all amniotic fluid samples were centrifuged at 3000xg for 20 min to separate cells from the supernatant, and then aliquoted and stored at −76 °C until use. The frozen samples were thawed at 20 °C before use. Amniotic fluid samples contaminated with blood or meconium were discarded.

The samples of amniotic fluid and plasma were divided into the following groups:

1) The second trimester, $n=21$: 14–18 weeks of pregnancy;
2) The third trimester, $n=12$: 35–37 weeks of pregnancy;
3) Perinatal period, $n=11$: 38–40 weeks of pregnancy;
4) Delivery, $n=40$: 38–42 weeks of pregnancy. Fetuses were delivered by spontaneous vaginal delivery within 3 h; and
5) Post-date pregnancy, $n=17$: 41–42 weeks of pregnancy. This group comprised of nine pregnancies terminated by cesarean section and seven pregnancies by spontaneous vaginal delivery lasting from 61 h to 200 h.

All women were healthy and delivered healthy newborns without malformations, chromosomal abnormalities, and postmaturity syndrome.

Additionally, plasma samples from healthy non-pregnant women ($n=15$) were included in the study.

Determination of immunoglobulin A concentration

Concentrations of IgA in the amniotic fluid and plasma samples were determined by conventional ELISA. In short, Maxisorp flat-bottomed 96-well microplates (NUNC, Roskilde, Denmark) were coated for 2 h at 37 °C, and overnight at 4 °C with 100 μl of 0.05 M carbonate/bicarbonate buffer (pH 9.6) containing 3 μg/ml of polyclonal rabbit IgA isotype-specific antibodies (DAKO, Glostrup, Denmark). After blocking with 1 % bovine serum albumin (BSA) (SERVA,

Heidelberg, Germany), 100 μl of twofold serial dilutions of women's plasma (range 1:10000–1: 80000), amniotic fluid (range 1:100–1:800), or IgA standard (range 0.0078–0.5 μg/ml) (Jackson Immunoresearch) in phosphate-buffered saline with 0.05 % Tween 20 (PBS-T) containing 1 % BSA, were added to each well and incubated for 2 h at 37 °C. After the washing step, 100 μl of horseradish peroxidase-conjugated rabbit antibodies diluted 1:4000 in PBS-T-1 % BSA were added to each well, and plates were incubated for 2 h at 37 °C. After washing, 100 μl of substrate solution containing ortho-phenylenodiamine (Calbiochem, Denmark) in 0.1 M citrate buffer, pH4.9 with H_2O_2 was added, and plates were incubated for 20 min at room temperature in the dark. The reaction was stopped with 1 M H_2SO_4, and optical density was read at $\lambda=490$ nm with a Dynatech MR 5000 photometric reader (DYNATECH, Dynex Technologies, Sullyfield Circle Chantilly, VA, USA).

Lectin-IgA-ELISA for differentiating sialyl- and fucosyl-glycovariants

Glycotope expressions on IgA were determined by lectin-IgA-ELISA, in accordance with the procedure described earlier, slightly modified, [14, 33] using specific biotinylated lectins [34] (Vector Laboratories Inc., Burlingame, USA): *Maackia amurensis* lectin (MAA) [28] and *Sambucus nigra* lectin (SNA) [29] with binding preferences to sialic acid linked by anomeric glycosidic α2,3 and α2,6 linkages and *Lens culinaris* lectin (LCA) [30], *Tetragonolobus purpureus* lectin (LTA) [31], and *Ulex europaeus* lectin (UEA) [32] showing binding preferences to fucose linked by anomeric glycosidic linkages α1,6, α1,3, and α1,2, respectively. However, the binding specificity of lectins is not absolute and usually covers a range of ligands with different relative affinities. Binding will therefore vary depending on the abundance and clustering patterns.

The experimental details of lectin-IgA-ELISA are as follows: rabbit specific anti-human IgA polyclonal antibodies (DAKO, Glostrup, Denmark), defucosylated and desialylated by oxidation with sodium periodate and further desialylated with *Vibrio cholera* neuraminidase, were diluted 1:1000 in 10 mM TRIS-buffered saline (TBS), pH8.5, coupled to a polystyrene microtitre ELISA plate, and used to extract and specifically bind IgA in the sample. For testing purposes, 100 μl of amniotic fluid or blood plasma samples, prediluted in 10 mM TBS pH7.5 containing 0.05 % Tween 20 to a final IgA concentration of 0.5 mg/l, were taken. The presence of the respective sialyl-glycotopes and fucosyl-glycotopes on IgA was detected by the reaction with the specific biotinylated lectins MAA (1:500) or SNA (1:20 000) and LCA (1:500), LTA (1:500), or UEA (1:500), respectively. The IgA-lectin complex was measured with phosphatase-labeled ExtrAvidin (1:20 000) (Sigma, St.

Louis, MO, USA), and then detected by the reaction with disodium 4-nitrophenyl phosphate (Merck, Darmstadt, Germany). The absorbance (AU) was measured in a Stat Fax 2100 Microplate Reader (Awareness Technology INC, USA) at 405 nm with the reference filter at 630 nm. Obtained absorbance values are proportional to the relative amounts/expressions of fucosyl- and sialyl-glycotopes of IgA.

All samples were analyzed in duplicate. The positive control for MAA, SNA, LCA, LTA, and UEA was native haptoglobin and asialo-haptoglobin preparations derived from ovarian cancer fluid [35]. The negative control was a human albumin preparation included in the test instead of the amniotic fluid and plasma samples. The background absorbance was low (0.05 ± 0.02 AU) when TBS was included in the tests instead of (1) lectin and (2) ExtrAvidin-AP.

Statistical analysis

The statistical analysis was performed with STATISTICA 10 (StatSoft, Inc., Tulsa, USA). The Kruskal–Wallis test and the Mann–Whitney U test were used for statistical significance. The results were shown as the mean ± SD. The correlations were estimated according to Spearman. A two-tailed p-value of less than 0.05 was considered significant.

Results

IgA concentration

The mean values of IgA concentration in the amniotic fluid (Table 2) and plasma of pregnant women (Table 3) showed no significant differences between the 2nd trimester, the 3rd trimester, perinatal period, delivery, and post-date pregnancy groups. The calculated mean and median values of IgA in all plasmas of pregnant women (1.9 ± 1.2 g/l and 1.8 g/l, respectively), corresponded to the normal range of IgA concentration in non-pregnant women (2.1 ± 1.3 g/l and 1.9 g/l, respectively), and were nearly 1000-times higher than in the amniotic fluids (7.7 ± 4.4 mg/l and 6.5 mg/l, respectively).

Table 2 Relative amounts of amniotic fucosyl- and sialyl-IgA glycoforms during normal pregnancy

No	Groups and number of samples	IgA mg/l	Amniotic IgA reactivity (AU) with lectins				
			SNA Specific to sialic acid attached by the linkages:	MAA	LCA Specific to fucose attached by the linkages:	LTA	UEA
			α2-6	α2-3	α1-6	α1-3	α1-2
1.	2nd trimester 14–18 weeks $n=21$	8.53 ± 2.8	0.66 ± 0.3	0.48 ± 0.3	1.04 ± 0.3	0.16 ± 0.1	0.24 ± 0.3
2.	3rd trimester 35–37 weeks $n=12$	6.86 ± 3.6	1.18 ± 0.5 [a]$p<0.001$	1.5 ± 0.5 [a]$p<0.00004$	1.11 ± 0.4	0.54 ± 0.4 [a]$p<0.002$	1.05 ± 0.7 [a]$p<0.0006$
3.	Perinatal period 38–40 weeks $n=11$	6.97 ± 4.0	1.15 ± 0.5	1.68 ± 0.4	1.01 ± 0.4	0.42 ± 0.4	1.0 ± 0.8
4.	Delivery at 38–42 weeks $n=40$	8.27 ± 4.9	1.06 ± 0.6	1.4 ± 0.6	0.98 ± 0.4	0.41 ± 0.4	0.75 ± 0.8
5.	Post-date pregnancy 41–42 weeks $n=17$	6.68 ± 4.3	1.42 ± 0.4 [b]$p<0.03$	1.69 ± 0.3	1.24 ± 0.3 [b]$p<0.01$	0.6 ± 0.3 [b]$p<0.009$	1.07 ± 0.6 [b]$p<0.03$

Values are given as the mean ± SD

The reactivity of 50 ng of IgA with MAA (*Maackia amurensis* lectin specific to α2,3-linked sialic acid), SNA (*Sambucus nigra* lectin specific to α2-6-linked sialic acid), LCA (*Lens culinaris* lectin specific to α1,6-linked fucose), LTA (*Tetragonolobus purpureus* lectin specific to α1-3-linked fucose), and UEA (*Ulex europaeus* lectin specific to α1,2-linked fucose) are expressed as the absolute value of absorbance (AU) at 405 nm based on the IgA-lectin immunoenzymatic assay as described in Materials and Methods

The Mann-Whitney U test and the Kruskal-Wallis test were used to calculate statistical differences between successive periods of pregnancy

A p-value less than 0.05 was considered significant. Significantly different from: [a] the 2nd trimester, [b] the delivery

Table 3 Relative amounts of plasma fucosyl- and sialyl-IgA glycoforms during normal pregnancy

No	Groups and number of samples	IgA g/l	Plasma IgA reactivity (AU) with lectins				
			SNA	MAA	LCA	LTA	UEA
			Specific to sialic acid attached by the linkages:		Specific to fucose attached by the linkages:		
			α2-6	α2-3	α1-6	α1-3	α1-2
1.	2nd trimester 14–18 weeks $n=21$	1.9±1.4	0.22±0.03	0.00	0.34±0.09	0.00	0.00
2.	3rd trimester 35–37 weeks $n=12$	2.0±1.1	0.22±0.04	0.00	0.31±0.08	0.00	0.00
3.	Perinatal period 38–40 weeks $n=11$	1.8±0.8	0.21±0.02	0.00	0.31±0.06	0.00	0.00
4.	Delivery at 38–42 weeks $n=40$	1.8±1.4	0.22±0.04	0.00	0.33±0.08	0.00	0.00
5.	Post-date pregnancy 41–42 weeks $n=17$	2.0±1.2	0.21±0.03	0.00	0.33±0.09	0.00	0.00
6.	Non-pregnant women $n=15$	2.1±1.3	0.23±0.04	0.00	0.31±0.06	0.00	0.00

Values are given as the mean ± SD

The reactivity of 50 ng of IgA with the respective lectin (for experimental details, see Table 1) is expressed as the absolute value of absorbance (AU) at 405 nm based on the IgA-lectin immunoenzymatic assay as described in Materials and Methods

The Mann-Whitney U test and the Kruskal-Wallis test were used for statistical calculations. A p-value less than 0.05 was considered significant. The mean values did not shown any significant differences between groups

Expression of MAA- and SNA-reactive glycotopes

The amniotic fluid IgA of all samples reacted with SNA and MAA indicating that the IgA glycans were potentially terminated with sialic acid linked by the α2,6 or α2,3 glycosidic bonds, respectively (Table 2). The expression of MAA-reactive glycotope on IgA showed weak positive correlation ($r=0.35$) with the age of normal pregnancy, but no correlation was found for SNA-reactive glycotope ($r=0.2$).

The relative reactivity of amniotic IgA with SNA (specific to α2,6-sialyl-glycoform) and MAA (specific to α2,3-sialyl-glycoform) was significantly lower ($p<0.001$ and $p<0.00004$, respectively) in the 2nd trimester group (0.66±0.3 AU and 0.48±0.3 AU, respectively) than in the 3rd trimester (1.18±0.5 AU and 1.50±0.5 AU, respectively). In the perinatal period (1.15±0.5 AU and 1.68±0.4 AU, respectively) and delivery (1.06±0.6 AU and 1.4±0.6 AU, respectively) groups, the sialylation were nearly at the same levels as in the 3rd trimester, however, α2,6-sialylation level became significantly higher in the post-date pregnancy group (1.42±0.4 AU; $p<0.03$) (Fig. 1).

The relative reactivity of plasma IgA of pregnant women with SNA (~0.22 AU) had not changed during the stages of pregnancy (Table 3) and was similar to that of non-pregnant women group (0.23±0.04), however being significantly lower ($p<0.00001$) when compared to that of amniotic IgA reactivity. In contrast to amniotic IgA, none of plasma IgA showed the reactivity with MAA (Table 2).

Expression of LCA-, LTA-, and UEA-reactive glycotopes

The amniotic IgA reactivity with LTA (specific to α1,3-linked fucose), UEA (specific to α1,2-linked fucose) and LCA (specific to α1,6-linked fucose) showed no correlation with the age of normal pregnancy ($r=0.16$; $r=0.17$ and $r=0.02$, respectively). In contrast, the expressions of all types of fucosylated glycotopes showed strong or very strong positive correlations ($r=0.68$ for UEA and LTA; $r=0.61$ for UEA and LCA; $r=0.89$ for LCA and LTA, respectively).

The amniotic fluid IgA from the 2nd trimester showed relatively high reactivity with LCA (for α1,6-fucose) and reacted slightly with LTA (for α1,3-linked fucose) and UEA (for α1,2-fucose) (Table 2). The reactivity of amniotic IgA with LCA remained nearly at the same level during the 2nd trimester (1.04±0.3 AU), 3rd trimester (1.11±0.4 AU), the

Fig. 1 Reactivity of amniotic fluid IgA with sialic acid-specific lectins in relation to pregnancy age. (**a**) with *Maackia amurensis* lectin; (**b**) with *Sambucus nigra* lectin. For experimental details, see Table 1. *Box* and *whisker plots*: the bottom and top of the box are the 25th and 75th percentile (the *lower* and *upper* quartiles, respectively), and the small box near the middle of the box is the median (the 50th percentile). The *ends of the whiskers* represent the minimum and maximum of all the data
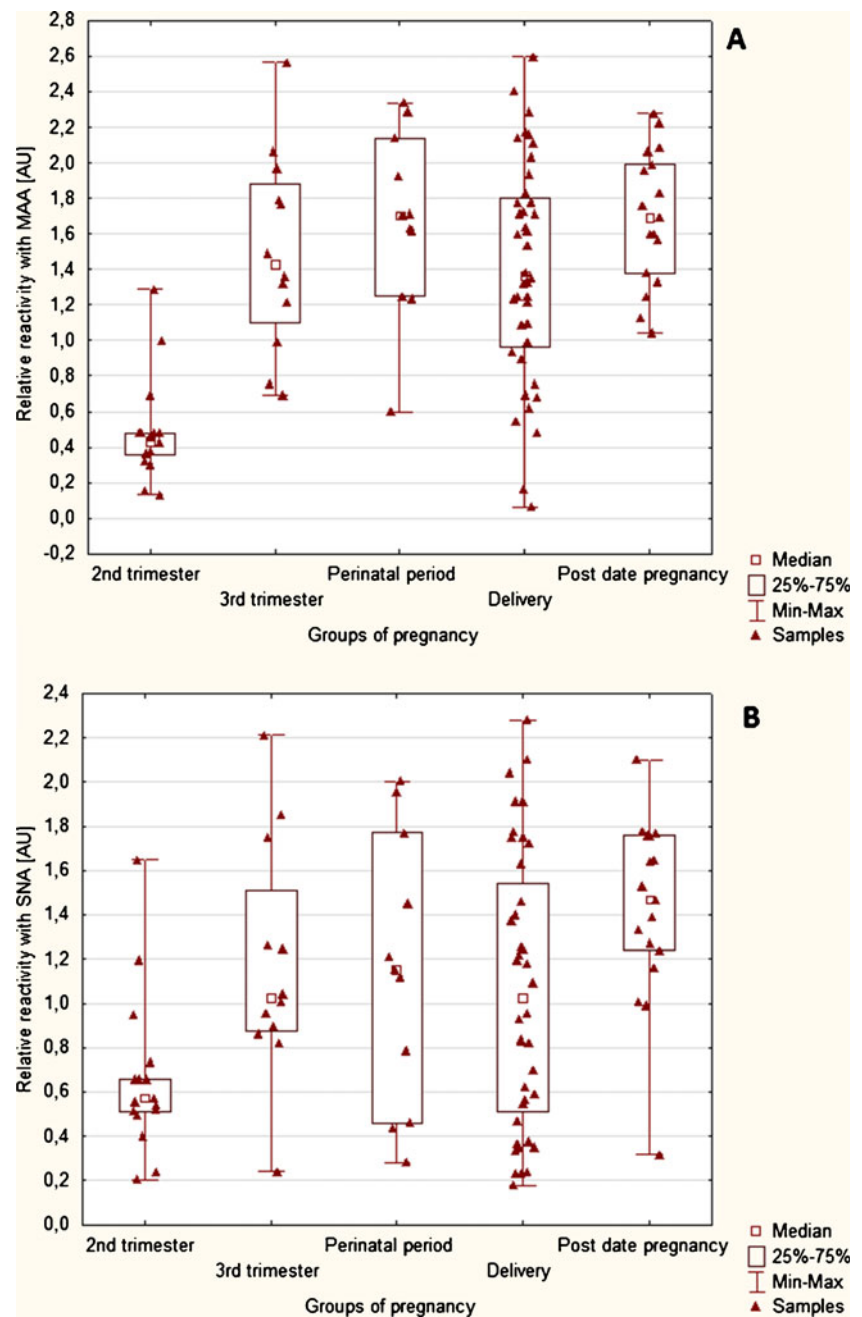

perinatal period (1.01±0.4 AU), and delivery (0.98± 0.4 AU). In contrast, the reactivity with LCA increased in the post-date pregnancy (1.24±0.3 AU; $p<0.01$). The significant increase of reactivity of amniotic IgA with LTA and UEA appeared in the 3rd trimester of pregnancy and remained nearly at the same level from the 3rd trimester (0.54±0.4 AU for LTA and 1.05±0.7 AU for UEA) throughout the perinatal period (0.42±0.4 AU for LTA and 1.0±0.8 AU for UEA) to the delivery (0.41±0.4 AU for LTA and 0.75±0.8 AU for UEA), though significantly increased in the post-date pregnancy (0.6±0.3 AU; $p<0.009$ for LTA and 1.07±0.6 AU; $p<0.03$ for UEA) (Fig. 2).

The relative amount of LCA-reactive glycotope of plasma IgA (Table 3) was at almost unchanged level throughout pregnancy of 14–42 weeks including the delivery group, and was three times lower when compared to amniotic IgA reactivity with LCA ($p<0.00001$). Plasma IgA lacked reactivity with α1-3- as well as α1-2-linked fucose specific lectins (LTA and UEA, respectively).

Discussion

Our results indicate that despite the almost unaltered concentration of IgA in amniotic fluid and pregnant women plasma,

Fig. 2 Reactivity of amniotic fluid IgA with fucose-specific lectins in relation to pregnancy age. (**a**) with *Lens culinaris* lectin; (**b**) with *Tetragonolobus purpureus* lectin; (**c**) with *Ulex europaeus* lectin. For experimental details, see Table 1. *Box and whisker plots*: the bottom and top of the box are the 25th and 75th percentile (the *lower* and *upper* quartiles, respectively), and the small box near the middle of the box is the median (the 50th percentile). The *ends of the whiskers* represent the minimum and maximum of all the data
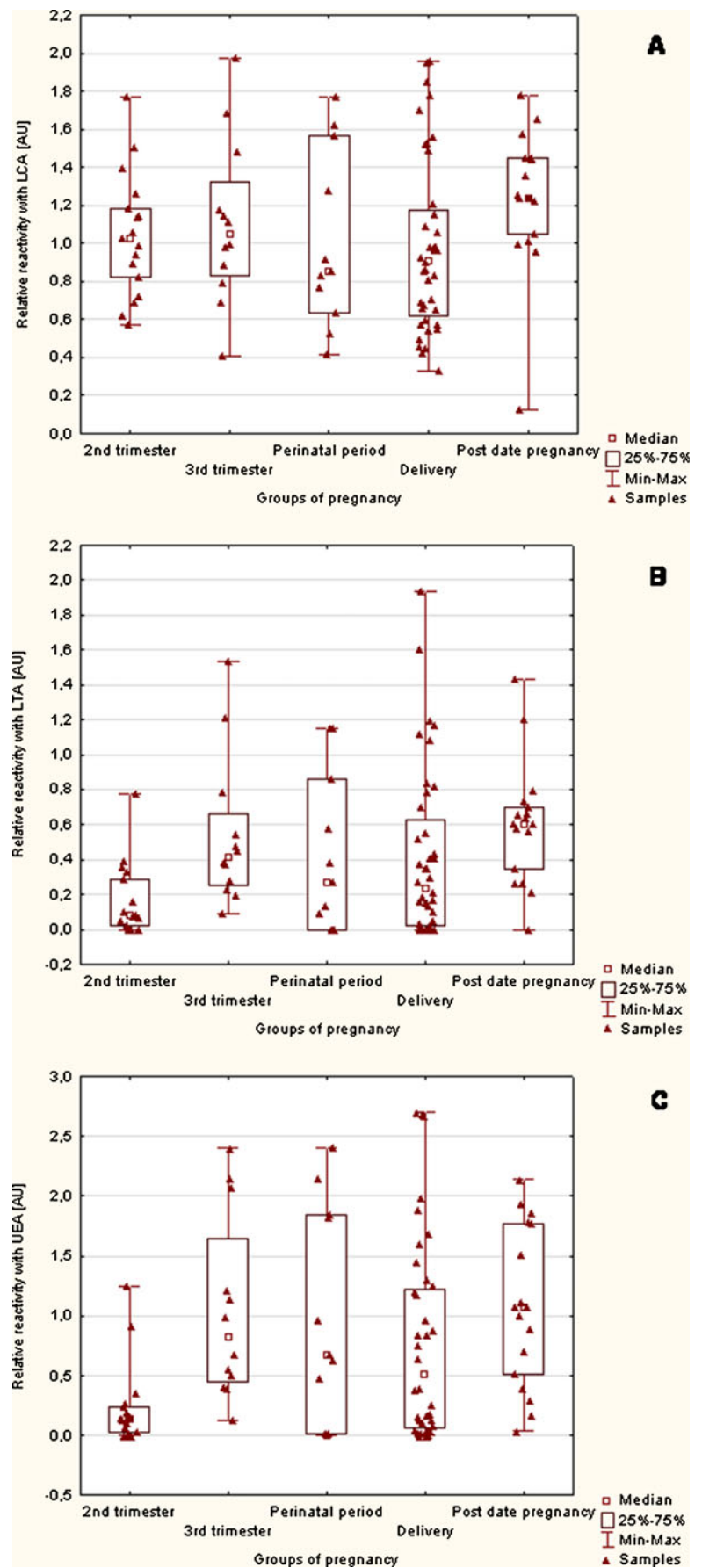
the degree of the relative sialylation and fucosylation of the amniotic IgA was connected with progression of the normal pregnancy.

Amniotic fluid might be considered a precursor of human milk feedings as an exogenous source of immunological factors that protect the infant directly and shape the responses of the infant's own immune system. Thus, the amniotic fluid being produced by, or at least transferred across, epithelial surfaces and bathes many of the mucosal surfaces of the fetus, might be considered part of the mucosal immune system [17]. Amniotic IgA might function as an "antiseptic coating" at the mucosal surface by preventing a bacterial adherence and an invasion of microorganisms [24].

In the amniotic fluid there is more than one source of IgA, namely maternal plasma, decidua and fetus. Maternal IgA is actively transported into the amniotic fluid and can be produced locally in the decidua, since Cederqvist et al. [36] reported that up to one-half of the IgA in amniotic fluid from 11 to 40 weeks of gestation was of the IgA2, characteristic for produced by mucosa [17]. The extent to which nonspecific diffusion and receptor-mediated transport of maternal IgA and the fetal mucosa contributes to amniotic IgA vary with gestational age [17, 19].

In the 2nd trimester of normal pregnancy the amniotic IgA showed a different pattern of sialylation and fucosylation than the plasma of pregnant women (Tables 2 and 3). The amniotic IgA was more highly LCA- and SNA- reactive than the maternal IgA, suggesting that amniotic IgA was heavily $\alpha2,6$-sialylated and $\alpha1,6$-fucosylated. Additionally, the amniotic IgA was decorated by glycotopes reactive with MAA, LTA, and UEA. The appearance of the $\alpha2,3$-sialylated glycotope on glycoproteins recognized by MAA was reported by Hampel et al. [37] to be bound with fetal development and classified as a fetal type of protein glycosylation. On the other hand, the plasma IgA of both pregnant and non-pregnant women did not show any reactivity with MAA (Table 3) and that observation shows a discrepancy with findings of Maenuma et al. [38]. They have reported a reactivity of mutated $\alpha2,3$-sialic acid-specific MAA with IgA O-glycans isolated from normal and IgA nephritis patients. It should be pointed out that in our lectin-based analysis, the lack of reactivity of the plasma IgA samples with MAA might result from a different spatial accessibility of $\alpha2,3$-sialylated glycotopes hidden in the structure of the hinge region of native IgA [26, 27, reviewed 39] of plasma sample compared to the isolated molecule of IgA. Moreover, the limited amount of native plasma and amniotic fluid IgA, namely 50 ng, used in our Lectin-ELISA probably also had impact on the observed lack of reactivity.

During the 3rd trimester, the last immunological phase of pregnancy, the fetus has completed its development; all the organs are functional and ready to deal with the external world [40]. Placental function is dynamic and influenced by maternal health, and has an important regulatory role in maternal and fetal well-being [41]. The glycosylation pattern of amniotic IgA seems to be a net result of maternal, fetal, and placental synthesis. Fetal urine is a major source, however, Goldblum and Hilton [17] suggest that the IgA is produced locally and excreted from the mucosal surfaces (other than urinary tract) of the fetus, namely respiratory, saliva and nasal secretions of fetus also contribute to the content of the amniotic fluid.

The presence of highly MAA-, LTA-, and UEA-reactive glycotopes at the turn of the 2nd and the 3rd trimesters is probable connected with fetal development and maturation. The fetus mucosal system production of IgA is increased and outweighs the mother's participation and has a direct impact on the glycosylation pattern of amniotic IgA during the 3rd trimester. The same characteristic quantitative switch was previously observed for amniotic IgG [14] and other amniotic glycoconjugates [42] and glycoproteins, namely $\alpha1$-acid glycoprotein [12] and fibronectin [43].

The expression of MAA-, SNA-, LCA-, LTA-, and UEA-reactive glycotopes on amniotic IgA has only slightly changed between the 3rd trimester and the perinatal period. Although the data are for limited amount of samples because of rarely occurring opportunities for the collection of amniotic fluid in this period, they are very valuable to understand the protective role of amniotic fluid. Moreover, the expression of LTA- and UEA-reactive glycotopes ($\alpha1,3$- and $\alpha1,2$-fucosylated glycans), which are known to create Lewis family determinants [2], valuable indicators of inflammation [44, 45] as well as molecules involved in innate immunity [25] may vary among individuals as a result of a non-secretor status [46] of mother and may reflect the sensitivity of individuals to external stimuli and efficiency of the immune system.

Delivery is characterized by an influx of immune cells into the myometrium in order to promote recrudescence of an inflammatory process. However, the proinflammatory environment which promotes the contraction of the uterus, expulsion of the baby, and rejection of the placenta [40, 47] seems to have no impact on sialylation and fucosylation of amniotic IgA during delivery. We have previously shown that during the delivery amniotic IgG [14] and fibronectin [43], glycoproteins which do not directly take part in response to inflammation (characteristic for acute phase proteins), also showed almost unaltered expression of fucosyl- and sialyl-glycotopes.

Fetal and neonatal mortality rates increase sharply after 40 weeks of pregnancy [48]. The pregnancy can be prolonged by inadequate hormone levels and maternal obesity and can increase the risk of Caesarean delivery and maternal complications. Around 41 weeks placental function may decline and become insufficient, reducing the supply of oxygen and nutrients to the fetus [41, 48]. We have observed that in the post-date pregnancy, there was the increase of the fetal or/and placental origin of highly fucosylated and
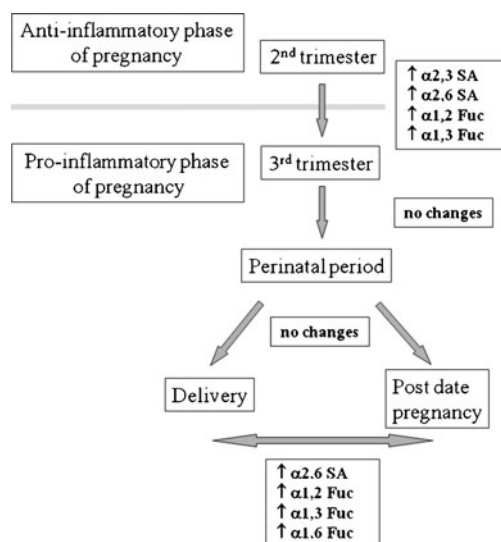
Fig. 3 The alternations of fucose and sialic acid expressions on amniotic IgA observed during pro-inflammatory and anti-inflammatory phases of the normal pregnancy. *SA* sialic acid; *Fuc* fucose

sialylated amniotic IgA glycoforms compared to those at delivery. The changes observed by us for amniotic IgA in the post-date pregnancy group, apart from MAA-reactive glycotopes, overlap/correspond to those reported for the amniotic IgG [14]. These findings can lead to the hypothesis that for amniotic immunoglobulins a common gestational age-dependent mechanism of glycosylation regulation can exist, probably connected with site-dependent synthesis and/or modulation of glycosylation.

In conclusion, pregnancy is a time of life where the development, maturation of fetus, and modulation of the immune system of mother, as well as differential responses of diverse biological events may occur based on glycotope-lectin receptor interactions. During the pregnancy stages these interactions might be important for controlling the pro-inflammatory and anti-inflammatory phases characterized by the unique inflammatory environment reported by Mor *et al.* [40]. In this aspect, the 1st and 3rd trimesters defined as proinflammatory phases (Th1) are responsible for the implantation and placentation as well as contraction of the uterus, expulsion of the baby and rejection of the placenta, respectively. On the contrary, the 2nd trimester represents an anti-inflammatory phase, also known as the Th2 environment, in which the mother, placenta and fetus are symbiotic [40, 49]. It should be noted that the observed changes of the expressions of lectin-reactive sialylated and fucosylated glycotopes at the turn of the 2nd and the 3rd trimesters also overlap with immunological phases of pregnancy (Fig. 3).

In our opinion, the pregnancy-associated unique expressions of sialylated and fucosylated glycoforms of amniotic IgA illustrate a general importance of carbohydrate-lectin and/or carbohydrate-carbohydrate interactions in the control

and modulation of pro- and anti-inflammatory events to ensuring the fetus well-being. Glycovariants of amniotic IgA associated with pregnancy progression overlap and/or are influenced by a gestational-depended pro- and anti-inflammatory condition. They seem to be molecular indicators reflecting the pregnancy age and can also be used as a window into the physiological events that lead up to both normal and post-date pregnancy. However, a future aim for these studies is the structural analysis of the IgA oligosaccharides that should confirm or verify relative levels of sialic acid and fucose of the lectin based proposals, and helps to understand their role and impact on the course of pregnancy.

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