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Placental Syncytiotrophoblast Maintains a Specific Type of Glycocalyx at the Fetomaternal Border: The Glycocalyx at the Fetomaternal Interface in Healthy Women and Patients With HELLP Syndrome

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Abstract

Recent studies showed that considerable amounts of glycosaminoglycans are released into maternal blood during normal pregnancy and in hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. Maternal endothelia and the syncytiotrophoblast layer have been discussed as a possible origin of these glycocalyx components. Our study aimed to visualize the glycocalyx on the syncytiotrophoblast by electron microscopy, to analyze its structure and composition by immunohistochemistry, and to determine potential differences between healthy women and women with HELLP syndrome. For electron microscopy, a cotyledon was fixed by perfusion of the intervillous space with a 2% lanthanum–nitrate glutaraldehyde solution followed by immersion fixation in the same fixative. For immunohistochemistry, sections of 16 placentas (HELLP patients/healthy women, n = 8 each) were stained with monoclonal antibodies against the main glycocalyx constituents syndecan 1, hyaluronic acid, and heparan sulfate. Semiquantitative evaluation of staining intensity focused on the apical surface of the syncytiotrophoblast and fetal intravillous endothelia as possible localizations of a placental glycocalyx. Electron microscopy revealed a glycocalyx of approximately 250 nm, covering the syncytiotrophoblast layer. This was found to contain large amounts of syndecan 1, but neither hyaluronic acid nor heparan sulfate as major components. Intravillous fetal endothelium did not express any of the investigated glycosaminoglycans. Healthy women and patients with HELLP showed no differences concerning glycocalyx composition and thickness of the syncytiotrophoblast. The composition of the "placental" glycocalyx differs from the adult and fetal vascular glycocalyx. Obviously, the human placental syncytiotrophoblast maintains a special kind of glycocalyx at the fetomaternal interface.

Keywords

glycocalyx, glycosaminoglycans, HELLP syndrome, placenta, syncytiotrophoblast

Introduction

A glycocalyx is assumed to cover the surface of all eukaryotic cells. In recent years, pronounced scientific efforts have been made to understand the structure and functionality especially of the endothelial glycocalyx (EGX). The EGX is involved in inflammatory processes and can be damaged by proinflammatory mediators like tumor necrosis factor-α (TNF-α), endotoxins, and oxidized lipoproteins.[1–5] In the vascular bed, the EGX serves as a regulatory interface as well as an important barrier between blood, interstitial space, and tissues. Concerning the placenta, this role has to be ascribed to the syncytiotrophoblast due to its epithelial nature and its direct contact with maternal blood in the intervillous space. Although a glycocalyx structure has not yet been visualized on the syncytiotrophoblast layer (STL), some molecular components constituting the EGX have already been detected in placental tissues, especially syndecan 1 and hyaluronic acid.[6–8] However, the term glycocalyx has not

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been mentioned in any publication investigating this subject. This may be due to earlier assumptions that considered the extension of the glycocalyx to be less than 50 nm and therefore beyond the resolution of light microscopy. However, recent investigations have revealed that formerly the thickness of the EGX has been severely underestimated and should be assigned between 0.5 and 1.0 μm.[9] In some investigations, it was even shown to be up to 2 μm.[10] A recent study of our group has revealed that considerable amounts of syndecan 1 are released into maternal blood with ongoing normal pregnancy.[11] Serum concentrations reached a median value of 10 500 ng/mL (week 38 of pregnancy), which proved to be more than 10 times higher than that in severe sepsis.[12] Elevated serum concentrations of 2 further glycosaminoglycans relevant for the EGX structure, hyaluronic acid and heparan sulfate, were only observed in women with the syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome). The maternal vascular bed, as well as the placenta, has been discussed as a possible source of these EGX components, but their exact localization remained unclear. Therefore, the current study focused on 3 goals: 1) to visualize a glycocalyx structure on the syncytiotrophoblast by electron microscopy and to evaluate whether this (putative) glycocalyx is thick enough to be detected by light microscopy and, thus, is suitable for immunohistochemistry; 2) to determine by immunohistochemistry whether the major components of the classical EGX also constitute the (hypothetic) trophoblastic glycocalyx or the EGX of the fetal intravillous vasculature; 3) to detect possible differences in the composition of the glycocalyx between healthy women and patients with HELLP syndrome.

Methods

The research protocol was approved by the institutional review board, and all women gave written informed consent before enrollment. Placental tissue was sampled from 8 healthy women (normal pregnancy group [NP]) and 8 women with verified class I or II HELLP syndrome (HELLP group [HG]), according to the Mississippi Triple Class System. This classification is based on platelet count nadir: class I: $<50 \times 10^9$/L; class II: 50×10^9/L to 100×10^9/L; class III $>100 \times 10^9$/L. Elevated liver enzymes (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] >70 U/L) and hemolysis have to be present in any case.[13,14] Patients with preexisting (prior to pregnancy) or gestational diabetes mellitus, preexisting arterial hypertension, vascular diseases, chronic or acute infections, or undergoing glucocorticoid therapy were excluded from the study as these conditions are known to impact the glycocalyx. None of the patients had been given heparin or heparin-like drugs.

Electron Microscopy

Electron microscopy was performed in a term placenta derived from a healthy woman.

Tissue preparation, fixation, and staining. For electron microscopy, a lanthanum–nitrate glutaraldehyde fixative was applied. A precondition for a successful lanthanum fixation is a constant flow of the fixative along glycocalyx-bearing cell surfaces for at least 30 seconds. This was achieved by the following procedure: immediately after delivery, the placenta was turned onto the fetal side. Two custom-made semicircular surgical clamps (diameter 5 cm) were positioned around a group of cotyledons situated at the placental margin. Thus, the encircled area extended from the placental margin about 5 cm in the direction of the umbilical insertion point, leaving a small section (about 1 cm) at the outer rim of the enclosed cotyledons for manipulations. Here a deep horizontal cut, about 1 cm wide, was made with a scalpel. Next, a needle attached to a 20-mL syringe was inserted vertically into the middle of the enclosed section and pushed forward until blood could be aspirated. Afterward, 40 mL of NaCl 0.9% solution were slowly injected into the enclosed cotyledons, washing out the intervillous blood via the preformed marginal cut. This procedure was followed by a slow injection of 20 mL of a freshly prepared solution of the lanthanum fixative, the composition of which is described in detail elsewhere, into the intervillous space to perfuse the syncytiotrophoblast surfaces.[9] Again, the overflow left the enclosed section via the marginal cut. After 5 minutes of latency to stabilize fixation, the enclosed section was removed from the placenta by sharp dissection along the clamps. Subsequently a horizontal dissection through the former enclosed cotyledons was made to reveal the fixed STLs and 4 small dices (each 1 mm^3) were obtained (1× perimarginal, 2× middle of the section, and 1× as near as possible to the middle of the placenta). Further fixation, washing, and contrast enhancement proceeded according to a protocol described by Chappell, in 2009.[9] After embedding in araldite and sectioning, electron microscopy (Philips CM, Aachen, Germany) was performed as previously described.[15]

Immunohistochemistry

Tissue preparation, fixation, and staining. Immediately after birth of the placenta, small lamellar tissue blocks including the fetal membranes were obtained by sharp dissection from the insertion point of the umbilical cord to the peripheral margin. Afterward, out of these stripes, 3 pairs of small blocks (about 9 mm^3) were excised near cord insertion, middle, and placental margin, respectively. Materials were obtained within 5 minutes after placental delivery and fixed in 4% formalin for 24 hours. Paraffin sections (5 μm) of every placenta were immunohistochemically stained with monoclonal antibodies against syndecan 1 (R&D Systems Inc, Minneapolis, Minnesota), hyaluronic acid (AbD Serotec MorphoSys GmbH, Germany), or heparan sulfate (Seikagaku Corp, Japan). Sections were incubated with 3% H_2O_2 for 30 minutes to inhibit endogenous peroxidase activity. Nonspecific antibody binding was blocked with 3% rabbit-0-serum or goat-0-serum (heparan sulfate) in phosphate-buffered saline for 30 minutes. The primary antibodies were diluted and pretreated with biotinylated secondary antibodies, corresponding to the initial blocking procedure.

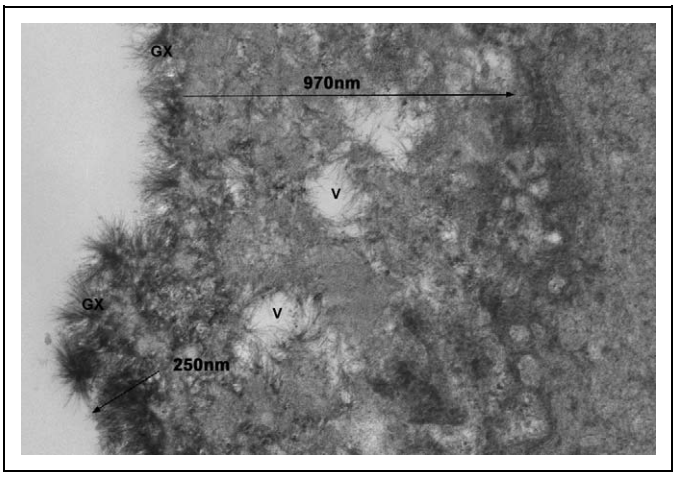

Figure 1. Electron microscopy of the apical surface of the syncytiotrophoblast layer after lanthanum fixation. Term placenta, undisturbed pregnancy. Original magnification: 30 000-fold. V indicates vacuoles covered with glycocalyx filaments; GX, glycocalyx.

Streptavidin–peroxidase conjugates (Vectastain-Kit, Vector Inc, Burlingame, California) were used to transfer peroxidase activity to the sites of antibody binding. Controls were treated identically, but the primary antibody was replaced with buffer (Figure 5A-F). Visualization was routinely achieved by peroxidase detection with diaminobenzidine as chromogen. Finally, all preparations were counterstained with hematoxylin.

Semiquantitative comparison of staining intensity. Preparations were examined by light microscopy using a 40-fold magnification. They were subdivided into 12 to 20 randomly selected, noncoherent fields of view, depending on the surface area of each section. Each field of view was digitally photographed. Afterward, the digital photographs were overlaid by a black grid subdividing them into 48 fields with the help of graphic software (ImageJ 1.44p; Wayne Rasband, National Institute of Health, USA). For each field, the intensity of staining was evaluated by 2 blinded investigators, using a modified version of the "h-score," with 0 = no staining, 1 = weak, 2 = mild, and 3 = strong staining.[16] Staining was only regarded as positive in case it occurred either on the apical surface of the STL or on the apical surface of the fetal intravillous endothelium. If the staining intensity varied within one field of the grid, the most intensively colored parts of the syncytiotrophoblast were evaluated and the highest score was noted. Thus, 29 840 fields were evaluated in total.

Statistics. Mean, standard deviation of the mean, median, and 25th/75th percentile were calculated for demographic and experimental data. To test normality, the Shapiro-Wilks test was applied. Because most of the data were not normally distributed, they are presented as median $\pm$ 25th/75th percentile. For intergroup comparisons concerning the intensity of immunohistochemical staining the Mann-Whitney Rank-Sum test was applied. For all determinations, a type I error protection of $P < .05$ was considered significant. Statistical analysis was performed using Sigma Stat Software version 3.1 (RockWare Inc, Golden, Colorado).

Results

Demographic and Clinical Data

The demographic data of the women enrolled and of their neonates are presented in Table 1. Except for gestational age at delivery and the body weight of the neonates, there were no significant differences between NP and HG.

Electron Microscopy (Healthy, Term Placenta)

A glycocalyx was visualized ex vivo on the surface of the syncytiotrophoblast of all analyzed fields. Although its structure was not preserved over the complete STL, large areas (about 50% of the syncytiotrophoblast surface) showed components of the glycocalyx structure in 2 areas, namely (i) at the apical surface of the syncytiotrophoblast in bush-like dense arrangements and (ii) intracellularly, predominantly with bush-like structures projecting into vacuoles in the intra-STL. No differences were observed between samples taken from different locations of the placenta (see methods section), either with regard to appearance or structure of the glycocalyx or with regard to the degree of damage and preservation, respectively. The intrasyncytiotrophoblastic zone reached a thickness between 600 and 1000 nm, the apical zone between 150 and 300 nm (Figures 1 and 2). Both zones together proved to extend between 900 and 1300 nm in diameter.

The filamental zone only partially resembled the structure of the glycocalyx discovered on human umbilical venous endothelium.[9,10] Filaments covering the syncytiotrophoblast surface were virtually grouped in bush-like entities, their tips clearly directed to the intervillous space. However, unlike the EGX, an apical cell membrane as a base for membrane-bound syndecans and transmembrane glypicans was barely differentiated. In the intrasyncytiotrophoblastic zone, numerous vacuoles were observed. These vacuoles were obviously filled with glycocalyx filaments. In this zone, discrimination between syncytiotrophoblast cytoplasm and glycocalyx material was difficult; the typical microvilli were hardly circumscribable.

In other fields of view, wide areas of the syncytiotrophoblast surface were covered by a broad margin (about 2-4 μm) of disrupted glycocalyx fragments and/or cellular debris, respectively (Figure 3).

Immunohistochemistry

Microscopical analyses focused on the apical surface of the STL and on the endothelium of intravillous fetal blood vessels. Positive reactions of other placental structures are not described and did not contribute to the findings concerning the intensity of immunohistochemical staining. The distribution of intensity score ratings is presented in Table 2.

Table 1. Demographic and Clinical Data of the Women Enrolled and Their Neonates.[a]

Groups (n)	HG (n = 8)	NP (n = 8)	*P* values, HG vs NP
Women			
Age, years	35 (34/36)	32 (31/35)	.15
Height, cm	165 (163/177)	168 (164/171)	.68
Body weight prior to pregnancy, kg	65 (60/71)	67 (65/74)	.42
Body weight after delivery, kg	72 (71/82)	74 (69/81)	.92
Gestational age at delivery, weeks	33.0 (31.2/35.5)	39.0 (37.0/39.5)	<.001
Cesarean section/spontaneous delivery, n	8/0	2/6	na
Neonates			
Body weight, g	1990 (1407/2512)	2935 (2672/3236)	.03
APGAR-score 1/5/10 minutes postpartum, points	8/10/10	9/9/10	.1/.2/.3
Arterial umbilical cord pH	7.31 (7.29/7.33)	7.33 (7.28/7.36)	.7
Arterial umbilical cord BE	−1.0 (−1.6/−0.1)	−2.0 (−3.9/−0.4)	.5

Abbreviations: na, not applicable; HG, HELLP group; NP, normal pregnancy group; HELLP syndrome, hemolysis, elevated liver enzymes, and low platelets syndrome.

[a] Data are given as median with 25th/75th percentile in parenthesis.

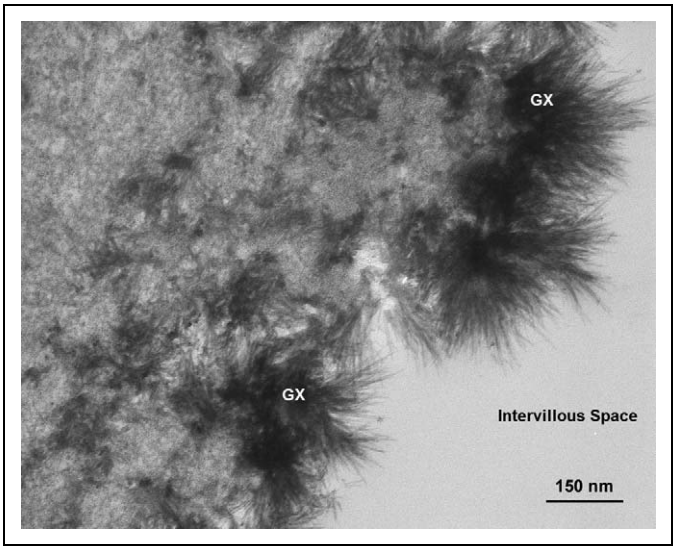

Figure 2. Electron microscopy of the apical surface of the syncytiotrophoblast layer after lanthanum fixation. Term placenta, undisturbed pregnancy. Original magnification: 49 000-fold. GX, indicates glycocalyx.

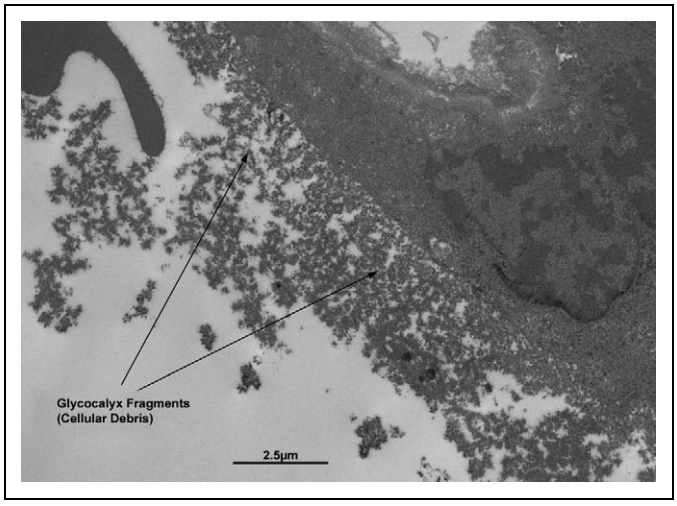

Figure 3. Human term placenta, luminal surface of the syncytiotrophoblast layer after lanthanum fixation. The syncytiotrophoblast layer is covered by disrupted glycocalyx fragments and/or cellular debris. Due to the perfusion technique, the glycocalyx of the fetal endothelium could not be imaged. Original magnification: 3000-fold.

Syndecan 1. Syndecan 1 was strongly expressed on the apical surfaces of the STL of all villi of normal, term placentas as well as of placentas gained from patients with HELLP (Figure 4A and D). More than 80% of the fields evaluated showed a score 3 reaction product (median modified H-score = 2.8 for both groups). Significant differences in terms of staining intensity between the HG and the NP could not be observed. The fetal endothelium remained completely unstained in all fields of view evaluated.

Hyaluronic acid. Compared to syndecan 1, the expression of hyaluronic acid on the apical surface of the syncytiotrophoblast was substantially less intense (median modified H-score = 0.6 for both groups). There were more negative staining reactions in the HG (HG 50.0% vs NP 39.3%), but definite statistical differences in terms of staining scores between the groups could not be noticed. In contrast to syndecan 1, hyaluronic acid sometimes could also be localized in the more basal parts of the syncytiotrophoblast, in the cytotrophoblast, and in basal membranes of the fetal endothelium (not shown). The luminal surface of the fetal endothelial layer did not demonstrate any positive reaction product (Figure 4B and E).

Heparan sulfate. There was hardly any positive expression of heparan sulfate on the STL in the investigated fields of view. A score 1 reaction product could be identified on the apical surface in less than 5% of the sections (median modified H-score = 0.0 for both groups). The endothelium of intravillous blood vessels remained unstained as well (Figure 4C and F).

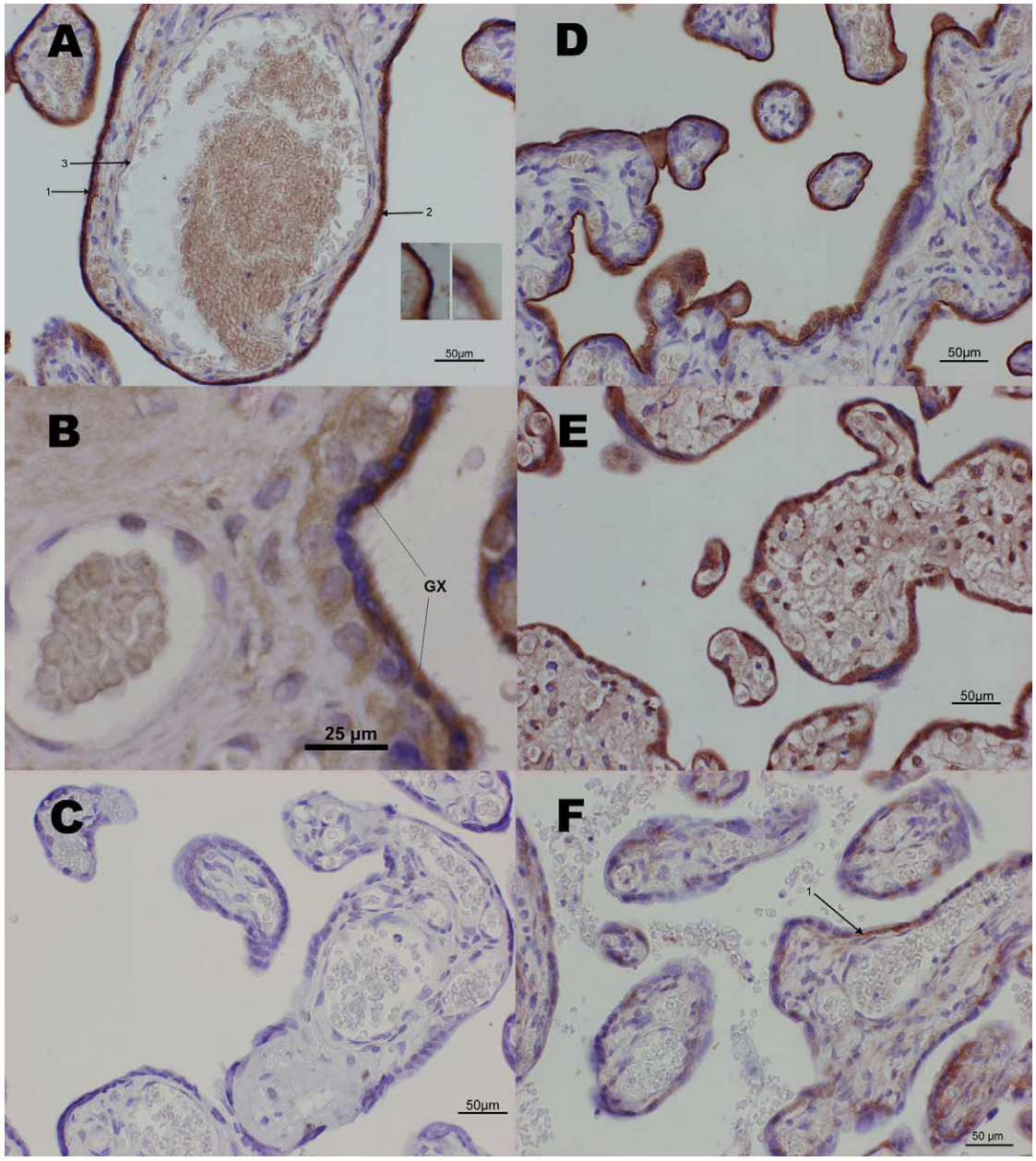

Figure 4. A-F, Immunohistochemical localizations of glycocalyx components on the syncytiotrophoblast layer. A-C, Normal term placenta, 39 weeks of gestation. A, Syndecan 1. Stainings presented themselves either as deeply brown to nearly black membranes (arrow 1; grade 3 H-score) or as slightly loosened layers (arrow 2). Small, magnified sections of another image of the same patient were integrated into this figure to give a more detailed view on these 2 types of staining reactions. The endothelium of fetal vessels remained completely unstained (arrow 3). Original magnifications: main picture 40-fold; magnified sections 80-fold. B, Hyaluronic acid. Grade 2 H-score. The endothelium of fetal vessels remained completely unstained. Original magnification: 40-fold. C, Heparan sulfate. The apical surface of the syncytiotrophoblast and the endothelium of fetal vessels remained completely unstained (grade 0 H-score). Original magnification: 20-fold. D-F, The placenta of patients with HELLP syndrome, 33 weeks of gestation. D, Syndecan 1. The black membranes and the slightly loosened layers (corresponding to Figure 4A) can also be identified. Original magnification: 40-fold. E, Hyaluronic acid. Grade 2 H-score. Original magnification: 40-fold. F, Heparan sulfate. Some positive staining reactions are detectable near the basal membranes of the syncytiotrophoblast (arrow 1) and in the cytotrophoblast. The apical surface of the syncytiotrophoblast and the endothelium of fetal vessels remained unstained (grade 0 H-score). Original magnification: 20-fold.

Discussion

The existence of a glycocalyx structure on the apical surface of the syncytiotrophoblast is evident, but, until now, neither has it been verified via electron microscopy nor has its molecular composition been clarified via immunohistochemistry. In the current study, a glycocalyx could be identified on the

Table 2. Intensity of Immunohistochemical Staining.[a,b]

	Syndecan 1	Hyaluronic Acid	Heparan Sulfate
HG			
Score 0, %	.0 (0.0/0.6)	50.0 (22.9/77.5)	100 (86.1/100)
Score 1, %	0.0 (0.0/2.9)	26.3 (18.4/42.1)	0.0 (0.0/13.9)
Score 2, %	10.0 (4.7/18.1)	7.9 (0.0/27.6)	0.0 (0.0/0.0)
Score 3, %	86.7 (76.3/94.7)	0 (0/0)	0.0 (0.0/0.0)
Median H-score	2.8 (2.7/2.9)	0.6 (0.2/1.0)	0.0 (0.0/0.1)
NP			
Score 0, %	0.0 (0.0/3.4)	39.3 (13.2/72.7)	97.7 (85.1/100)
Score 1, %	0.0 (0.0/10.0)	29.0 (16.0/45.6)	2.3 (0.0/14.5)
Score 2, %	12.1 (3.8/20.9)	10.3 (0.0/30.9)	0.0 (0.0/0.0)
Score 3, %	83.0 (62.6/95.1)	0.0 (0.0/2.6)	0.0 (0.0/0.0)
Median H-score	2.8 (2.4/2.9)	0.6 (0.3/1.3)	0.0 (0.0/0.2)

Abbreviations: HG, HELLP group; NP, normal pregnancy group; HELLP syndrome, hemolysis, elevated liver enzymes, and low platelets syndrome.
[a] Data are shown as median (25th/75th percentile).
[b] Significant differences between groups could not be observed, $P > .05$ for all comparisons.

syncytiotrophoblast surface by applying a new method of perfusion fixation for placental electron microscopy. In addition, with up to 1100 nm in size this glycocalyx was found to be suitable for light microscopy. Despite these basic observations, some additional ultrastructural features of the syncytiotrophoblast's glycocalyx could be described. However, these findings have to be carefully interpreted due to the fact that only one placenta was investigated by electron microscopy. Electron microscopy as well as immunohistochemical staining suggests that the composition of the "placental" glycocalyx differs from the EGX of adult humans.

Concerning its ultrastructure, especially the "nonexistence" of a visible apical cell membrane contrasts to that of EGX. Although the number and length of the placental microvilli are reduced with ongoing pregnancy, it is likely that the nonlinear surface pattern of the syncytiotrophoblast prevents the formation of a "straight line" continuous glycocalyx and, thus, conceals the apical cell membrane of the STL.[17]

Various investigators have tried to evaluate the thickness of the EGX.[4,15,18–20] Today, the extension of the EGX is thought to be up to 0.5 μm, some investigators even measured 2 μm by confocal laser scanning microscopy, depending on the location within the vascular system.[10,21] With a thickness of about 250 nm, the glycocalyx covering the STL in the current study seems to be half the size of the EGX of major blood vessels and, at first sight, at the limit of visibility when investigated via light microscopy. However, in contrast to vasculature, placental glycocalyx filaments were not only localized along the STL's surface, but a dense "inner zone," corresponding to numerous intrasyncytiotrophoblastic vacuoles (or perhaps invaginations), could also be identified (extension about 1100 nm), in which glycocalyx filaments seemed to be embedded while "carpeting" the inner surface of these vacuoles. Vacuoles or vesicles in between the syncytiotrophoblast stroma have been described by various authors; their functions have not yet been fully understood.[17,22] Haigh et al thought it is possible that some of the apical vacuoles discharge their contents into the intervillous space and afterward function as a type of residual membranous body.[23] Despite the fact that the synthesis of the glycocalyx is not well understood, one can speculate that glycocalyx-covered vacuoles can migrate to the STL's surface and may be integrated into its apical membrane with its glycocalyx-covered surface directed to the intervillous space.

The fact that glycocalyx filaments—at least in the current experiment—are localized on the surface as well as in a 1 μm broad zone of the cytoplasm of the syncytiotrophoblast, is in accordance with the immunohistochemical findings; syndecan 1 can be detected not only on the apical surface (where it displayed the highest staining intensity), but also in the syncytiotrophoblast plasma in the current study, as well as in previous investigations (Figure 4A and D).[6,7] Besides large areas covered by an intact glycocalyx, in the current study, other parts of the STL displayed a surface clad only by disrupted glycocalyx fragments and/or cellular debris. Due to the limitations of ultrastructural imaging, the current investigation is not appropriate to decide whether this is physiological and only reflects an enormous cellular turnover (what would correspond to high maternal serum levels of glycocalyx components[11]), or has to be interpreted as an unnatural damage due to the beginning of tissue hypoxia after delivery. The lanthanum-perfusion technique applied here perfused only the intervillous space. Thus the EGX presumably lining fetal vessels escapes representation from electron microscopy. However, the existence of a fetal EGX has already been confirmed by an ultrastructural investigation performed by Eaton et al in 1993.[24] With immunohistochemistry both the fetal (endothelial) and the maternal (syncytial) side of the feto-maternal interface, can be analyzed.

Indeed, syndecan 1, heparan sulfate, and hyaluronic acid have already been detected in a variety of localizations in the human placenta.[6–8,25–29] Unfortunately, the results of previous studies vary both for the local expression and for staining intensity of these components in placental tissues. Their expression has also been investigated in the context of different pregnancy disorders, but quantitative comparisons of immunohistochemical staining intensity are generally rare, and patients with HELLP syndrome have not yet been investigated.[6,29] The current study adds new aspects to the ongoing discussion on glycosaminoglycans in placental compartments by focusing on the glycocalyx of the placenta's epithelial structures: the syncytiotrophoblast and the endothelium of the fetal intravillous vasculature.

Concerning syndecan 1, data provided by the current study confirm and amend the results of Crescimanno and coworkers who noticed a strong expression along the apical plasma membrane of the syncytiotrophoblast.[6] Comparative investigations on the staining intensity of syndecan 1 on the STL's surfaces in patients with HELLP patients are not available, but Jokimaa et al reported a reduced syndecan 1 expression on the chorionic villi in severe preeclampsia.[29] This discrepancy to the current investigation is difficult to interpret, because Jokimaa et al did not exclusively refer to the STL.

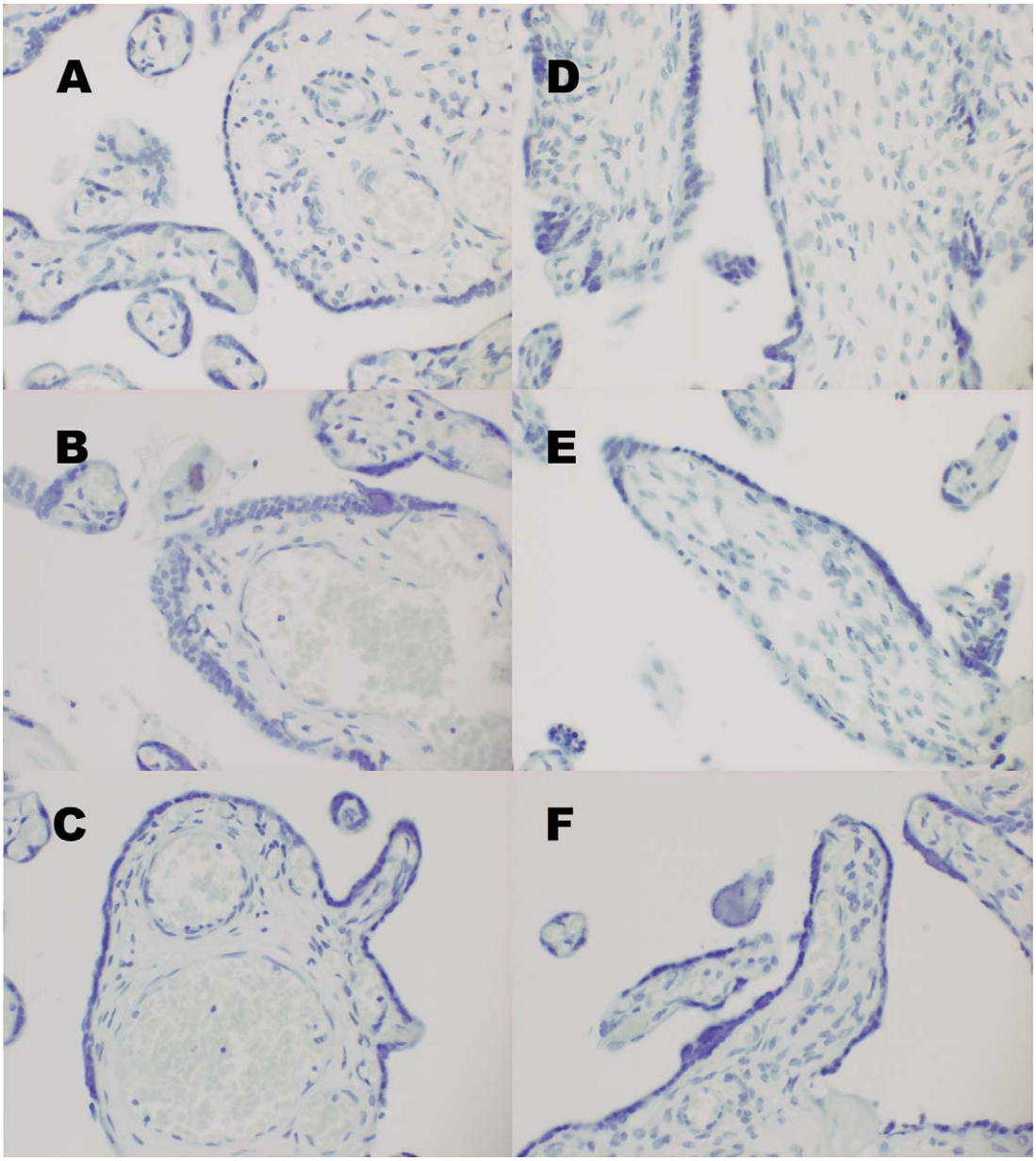

Figure 5. A-F, Negative controls. A-C, Normal term placenta, 39 weeks of gestation. A, Syndecan 1. B, Hyaluronic acid. C, Heparan sulfate. D-F, The placenta of patients with HELLP syndrome, 32 weeks of gestation. D, Syndecan 1. E, Hyaluronic acid. F, Heparan sulfate. Original magnifications 5A-F: 40-fold.

In addition, it is still unclear whether study results obtained from women with preeclampsia are transferable to patients with HELLP. Nevertheless, the equal staining intensities of syndecan 1 on the STL in NP and HG speak in favor of the assumption that the large amount of syndecan 1 measured in the blood of patients with HELLP, which exceeds the elevated levels measured in healthy women, might at least partially derive from the maternal vasculature.[11]

With respect to intravillous fetal endothelium, we did not notice any positive reaction products for syndecan 1. This again is in accordance with the results provided by Crescimanno et al who also noted the absence of syndecan 1 but found syndecan 2 in the walls of fetal vessels, particularly in the endothelium in the 9th week of gestation.[6] This is remarkable, because Chappell et al were able to detect syndecan 1 in the umbilical veins and arteries of term placentas.[9] It is unclear whether syndecan 1 expression patterns can change with ongoing pregnancy (what seems possible according to Crescimanno's studies), or whether the composition of the EGX of chorionic endothelia principally differs from the EGX of fetal umbilical vessels. The results concerning hyaluronic acid and heparan sulfate provided by the current investigation do speak in favor of the latter possibility.

Regarding hyaluronic acid, weak to moderate staining was detectable on the apical surface of the STL in 50% (NP) and 39% (HG) of the sections investigated. In 2010, Gao et al reported that enzymatic degradation of hyaluronic acid reduces the thickness of the EGX by 26%, indicating that hyaluronic acid is not the predominant glycosaminoglycan in the EGX.[30] Although certainly not directly comparable, these findings do correspond to our results concerning the weak to moderate staining intensity of hyaluronic acid on the STL we observed. Surprisingly the fetal endothelia displayed no staining. There is one comparable study performed by Matejevic et al who observed hyaluronic acid in the fetal vessel walls (tunica intima, media, and adventitia), but the authors unfortunately did not refer to the endothelium.[8]

For heparan sulfate, the third major component of the EGX examined in the current study, only minimal traces of reaction products could be detected on the STL and none on fetal endothelia. Again no relevant difference existed between patients with HELLP and healthy women. Older studies are not completely in accordance with these results. For example, Wasserman et al observed that heparan sulfate was associated with intravillous blood vessels and the syncytiotrophoblast but did not provide data concerning the intensity and exact localization of their findings. Placental tissue obtained from patients with pregnancy disorders was not examined in Wasserman's investigation.[31]

The current study focused on healthy women and patients with HELLP syndrome and did not include women with preeclampsia. In addition, we did not investigate placentas gathered from women whose prematurity (see Table 1) was not caused by a HELLP syndrome. This might be considered as a limitation. However, although preeclampsia is well defined by international protocols, these protocols usually leave space for a variety of clinical manifestations and are partially independent from the severity of the disease.[32,33] Integrating women with preeclampsia probably would have led to an inhomogeneous study population and, thus, complicated the interpretation of results. In contrast, HELLP syndrome is unmistakably defined by a typical set of laboratory values, the women concerned definitely display severe clinical symptoms and the disease is clearly associated with inflammation.[14,34] In terms of prematurity, inflammation is supposed to account for the bigger part of preterm deliveries.[35–39] Even in cases without obvious inflammatory background (eg, cervix insufficiency), inflammation is considered to be a conductive factor.[38] However, the most important causes of glycocalyx destruction are inflammation and ischemia.[1,12,40] Thus, until the origin of prematurity is completely understood, "normal" (non-HELLP) preterm placentas would have led to an unpredictable integration of factors influencing the glycocalyx's structure and integrity; perhaps preterm delivery per se, surely inflammation and probably ischemia. This again would have hindered a reasonable interpretation of study results. Accordingly, it seemed advantageous to include only women with HELLP syndrome in the current study and, as a consequence, to compare 2 well-defined groups of pregnancies with and without an inflammatory background, even if this led to relatively small study groups.

Conclusion

Summarizing the results of immunohistochemistry and electron microscopy, one can state that the glycocalyx detected on the STL obviously deviates from the EGX with regard to the composition of its major components and its structure. Especially the scarcity of heparan sulfate on the apical surface of the STL emphasizes the constitutional differences to the adult EGX and, on the other hand, supports the hypothesis that elevated heparan sulfate and hyaluronic acid serum concentrations in women with HELLP syndrome do not derive from the placenta, but rather from the maternal vasculature (or other origins). HELLP syndrome, as an inflammatory disorder, did not influence the results of semiquantitative immunohistochemistry. Concerning the EGX of fetal vessels, the findings of the current study only partially correspond to previous investigations. Further research is required to exactly define the composition and structure of this side of the fetomaternal barrier.

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