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Transient CD15-positive endothelial phenotype in the human placenta correlates with physiological and pathological fetoplacental immaturity

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ABSTRACT

Objective: Placental growth and villous maturation are critical parameters of placental function at the end of pregnancy. A failure in these processes leads to the development of placental dysfunction, as well as fetal and neonatal mortality and morbidity. The aim of the study was to determine the relevant diagnostic markers associated with pathological placental development.
Study design: Forty tissue samples from normal placentas of different gestational age and 68 pathological term placentas with defective villous maturation (GDM, idiopathic IUFD, preeclamsia, HELLP syndrome) comprised the comparative immunohistochemical study (CD15, CD45 and CD34). Positive immunohistochemical reactions were quantitatively assessed in the chorionic plate and vessels of the villi of different histological type.
Results: Physiologically immature placentas of the first and second trimester and pathologically immature term placentas were characterized by marked endothelial CD15-immunostaining. A significant loss of CD15-positive endothelium of the placentas was associated with a physiological and accelerated villous maturity. A spatio-temporal correlation was shown for CD15+ endothelial cells (ECs) and the number of CD45+ stromal cells (SCs). A negative temporal correlation was shown for CD15+ ECs and CD15+ myelomonocytes in the fetal blood. CD34 expression in the ECs was stable during the pregnancy.
Conclusion: A correlation between a transient CD15-positive endothelial phenotype and a physiological and pathological fetoplacental immaturity was demonstrated. Physiological and accelerated placental maturation was accompanied by a significant disappearance of CD15-positive endothelium. We propose that "immature" CD15+ endothelium is an important diagnostic marker of the physiological and pathological fetoplacental immaturity.

Introduction

Placental growth and villous maturation are critical parameters of placental function at the end of pregnancy [1]. A failure in these processes leads to the development of placental pathologies, placental dysfunction, fetal and neonatal mortality and morbidity [1–4]. Reliable diagnostic tests of placental pathologies remain a major clinical challenge [4].

Placental growth and villous development undergo significant changes during a physiological pregnancy [1]. Physiological immaturity in the first and second trimester is characterized by the development of the immature villi which provide placental growth [1,2]. Physiological villous maturation in late pregnancy (third trimester) is accompanied by restriction of the growth potential and primary development of terminal villi providing the respiratory capacity of the placenta [1,5].

Two main contrasting types of developmental disturbances of placental growth and maturation in the third trimester—accelerated and delayed maturation—have been described [1,2,6]. Pathologically accelerated villous maturation is characterized by a deficiency of immature villi with growth restriction and a predominant differentiation of terminal villi [1,2]. Maternal

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preeclampsia, HELLP syndrome and about 50% of preterm deliveries are associated with this defect of maturity [1,2].

Pathologically delayed maturation or persisting villous immaturity is characterized by a predominance of immature villi, intensive growth potential and a deficiency of terminal villi [1,2]. The etiology and pathophysiology of villous immaturity are unknown. Important risk factors are gestational diabetes mellitus (GDM), viral diseases and postterm pregnancy [1–3]. About 50% of cases are idiopathic villous immaturity with a latent course of the placental insufficiency and risk of an unexpected antenatal decompensation involving fetal hypoxia (38%) and intrauterine fetal death (IUFD, 9%,) [1–3].

The fetoplacental endothelium is an essential component of placental development. Furthermore, phenotypic changes in the endothelium at different gestational ages may affect fetoplacental development [7].

In the past years increasing evidence indicates that hematopoietic function of the placenta in the presence of the phenotypic peculiarities of placental endothelium is related to the presence of transient placental hematopoiesis [8–10]. The role of endothelium in the fetoplacental interactions and maturation is still unclear.

Endothelial CD15-expression is a unique phenomenon described in the fetoplacental endothelium and coincides in time with the expression of placental hematopoiesis [10–12]. CD15 (called Lewis X and stage-specific embryonic antigen 1) is a carbohydrate adhesion molecule that can be expressed on glycoproteins, glycolipids and proteoglycans [13,14]. CD15 acts as a key ligand of selectin and plays an important role in adhesion, migration and differentiation of the cells in the embryo–fetal tissues. CD15 was identified in stem cells, myeloid cells, mature blood cells and tumor cells [14–20]. CD15 is expressed in some tissues, such as epithelial cells of intestinal tissues, certain neurons and glial cells in the central nervous system. In human leukocytes, CD15 is expressed preferentially in monocytes, mature neutrophils and all myeloid cells from the promyelocyte stage onwards [21].

The aim of the study was to determine the relevant diagnostic marker associated with pathological placental development.

Materials and methods

Tissue specimens and histology

Hundred and eight tissue samples were obtained from normal and pathological placentas of different gestation age, which underwent examination in the Department of Pathology, University Mainz from June 2010 to September 2013. Gestational ages were checked by ultrasound, as well as by clinical and morphological examination. The main clinical and morphological data of the placentas are given in the Table 1.

Excess placental material was used in these studies in accordance with the regulations of the Local Ethical Committee. In all cases, advanced written informed consent was obtained from the pregnant women for tissue used in these studies.

Placental tissues were fixed in 4% neutral buffered formalin. Macroscopic and histological observations of the placenta were performed according to the Vogel principles (1996) [5]. Histological criteria of villous development and maturation were the degree of branching, stromal differentiation, vascularisation and the formation of syncytiocapillary membranes [1,5].

All normal placentas were without any macroscopic or histological abnormalities. Placentas with pathological villous maturation were divided into 2 groups: placentas with persisting villous immaturity and a deficiency of terminal villi; placentas with accelerated villous maturation, a deficiency of immature intermediate villi and an increased differentiation of the terminal villi.

Immunohistochemistry

For immunohistochemistry, 3 μm sections were deparaffinized, rehydrated and washed with PBS. An automated immunostaining was performed (Autostainer®, DAKO) using a labeled streptavidin-biotin immunoenzymatic antigen detection system (DAKO EnVision, System- HRP mouse/rabbit) according to the manufacturer's instructions. 3,3-Diaminobenzidine tetrahydrochloride was used as chromogen.

The following antibodies were used. Mouse monoclonal IgM against human CD15 (Ready-to-Use, DAKO, clone Carb-3). Mouse monoclonal IgG against human CD34 (Ready-to-Use, DAKO, clone QBEnd 10; antibodies reacts with human hematopoietic progenitor cells, including myeloid and lymphoid progenitors, labels capillaries of most tissues [22]). Mouse monoclonal IgG against human CD45 (Ready-to-Use, DAKO, clones 2B11 and PD7/26; interacts with all hematopoietic cells from HSCs to mature blood cells [23]). For negative controls, the primary antibodies were substituted with the PBS.

Immunohistochemical reactions of endothelial and stromal cells were differentially assessed in the chorionic plate and in the vessels of the villi of different histological type. Stained vessels were counted per 100 vessels of the chorionic plate, stem villi, immature and mature villi. Their ratio was calculated as percentage of positively stained vessels. Positive reaction of ECs in the vessel was graded as: "++" all ECs of the vessel were stained; "+" individual ECs were stained. Staining of the SCs was following: "−" 0 cells high-power fields (HPF), "+" 1–10 cells per HPF, "++" more than 10 cell per HPF, "+++" more than 20 cell per HPF.

Table 1
Main clinical and morphological data of the placentas.

	Physiological villous immaturity		Physiological villous maturity	Pathological persisting villous immaturity	Pathological accelerated villous maturation
Trimester (N)	I (14)	II (12)	III (14)	III (54)	III (14)
Gestation age (weeks)	8–11	16–21	40–41	38–40	38–40
Weight (g)	Not define	170 ± 47	420 ± 46	590 ± 31	371 ± 100
Clinical diagnosis	Abruptio (14)	Late abortion (6), Cervical incompetence (6)	Spontan Partus (14)	Antenatal fetal hypoxia (54) GDM (20), idiopathic IUFD (5)	Preeklampsia (8), HELLP (6)
Pathological cardio-tocography in antepartum	Not define	Not define	0	54	11
Caesarean section	0	0	0	12	6
Fetal development	Norm	Norm	Norm	Macrosomia (4)	IUGR (5)
Infections	No	No	No	No	No
Embryonic/Fetal malformations	No	No	No	No	No
Features of an umbilical cord	No	No	No	No	No

GDM—gestational diabetes mellitus, IUFD—Intrauterine fetal death, IUGR—Intrauterine growth restriction