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The molecular changes during placental detachment

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Abstract

Objectives: RCAS1 is a membrane protein that plays a role in the maintenance of maternal immune tolerance during pregnancy. The work presented here demonstrates the results of RCAS1 expression in placenta in cases of placental abruption and patients with retained placental tissue during the third stage of labor.
Study design: The placenta tissue samples were obtained during vaginal and cesarean delivery (derived from 117 pregnancies). Pregnant women were divided into four groups according to the onset of labor and the time of placental detachment in term labors. The samples were analyzed by the Western blot method. Statistical analysis was performed using the Shapiro–Wilk procedure. The Mann–Whitney test and Student's *t*-test were applied to compare the differences between parametric data.
Results: The average relative amount of RCAS1 observed in those patients with retained placental tissue was statistically significantly higher than in the patients with placental abruption.
Conclusion: The differences observed in placental RCAS1 levels confirm the participation of this protein in the inhibition of maternal immune response during gestation. The present results also indicate that RCAS1 participates in the changes in the maternal immune system that take place during parturition and reinforce its potential involvement in the mechanism of placental abruption.

Keywords: Immune tolerance; Placental abruption; RCAS1

1. Introduction

Pregnancy involves immunological interactions between the maternal immune system and the fetus. Fetal development depends on the proper level of immune tolerance during pregnancy. The termination of a normal pregnancy is clinically observed as a spontaneous labor and is accompanied by an increase in the cytotoxic activity of lymphocytes [1]. The immune system of the female reproductive tract is unique and is restricted by hormonal changes. The human uterus contains the full range of immune cells. Decidual natural killer (dNK) cells are also present in the uterine endometrium, where their number increases during the late secretory phase and accounts for up to 70–80% of lymphocytes in the first trimester of pregnancy. Endometrial monocytes and macrophages are distributed diffusely throughout the uterine stroma in relatively high numbers and represent 5–15% of the endometrial stromal cells [2,3]. Lymphoid aggregates (LA) are unique and organized structures among the lymphocytes that are found in the endometrium. They consist of a core of B cells surrounded by $CD8^+$ T cells and an outer halo of monocytes and macrophages [3,4]. Recently, the existence of NKT cells, which express both NK and CD3 T cells markers, was confirmed in the endometrium [5].

Activation of lymphocytes in the decidua is mediated by cytokine production. This regulation is controlled by T helper cells, macrophages and decidual stromal cells (DSC) [6]. Pregnancy is associated with a relative increase in Th_2-dependent immunity, characterized by increased production of the cytokines IL-4 and Il-10 with a concomitant decrease in Th_1 action (IL-2, IL-12 and INF-γ [7]. As a consequence, the increase in Th_1 activity has been shown to induce

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pregnancy termination [1]. The rise in Th_2 immunity is not sufficient to create an efficient level of immune tolerance. A recent investigation concerning immune tolerance revealed the possible role of receptor-binding cancer antigen expressed on SiSo cells (RCAS1) in the maintenance of pregnancy [8]. This is a type II membrane protein, expressed in extra-villous cytotrophoblasts, villi-histiocytes, uterine endometrium, and in various human cancer cells [9–12]. RCAS1 protein acts as a ligand for a putative receptor present on normal peripheral lymphocytes such as T, B, and NK cells. RCAS1 inhibited the growth of receptor expressing cells in vitro and in vivo and induced apoptotic cell death [9,13]. Functions of RCAS1 include avoiding immune recognition and evading immune surveillance. RCAS1 may participate in the inhibition of the maternal immune attack on the fetal antigen [14,15]. The aim of our research was to evaluate the changes in the placental level of RCAS1 during the process of placental detachment in the course of labor.

2. Materials and methods

2.1. Subjects

We included in our study 117 patients. The material was collected in the Department of Gynecology and Infertility Clinic of Jagiellonian University during both vaginal and cesarean deliveries at term. Patients with multiple pregnancies or existing pregnancy complications such as preterm deliveries, hypertension, diabetes mellitus, and cases of fetal demise were excluded from this study.

2.2. Selection of pregnant women

We divided the patients from whom our material was derived into four groups, according to the type of labor and the type of placental separation. The first group included women in whom emergency cesarean section was performed due to placental abruption during the first stage of labor. The indications for an emergency cesarean section in these patients were as follows: rapidly developing uterine tenderness, fetal distress syndrome and/or severe vaginal hemorrhage. In all these cases, a retroplacental clot was confirmed following the surgical procedure.

The second group consisted of women who delivered vaginally and in whom retained placental tissue was found following the third stage of labor. Curettage was performed in all these patients in order to avoid further complications such as bleeding and infection. The third and the fourth groups were the control groups and were selected according to the type of labor. The third group represented women with vaginal delivery of spontaneous onset with regular uterine contractions in the first and second stages of labor. In all these women, the placenta detached normally in the third stage of the labor. The fourth group included cesarean deliveries performed with an unripe cervix and without uterine contractions (elective cesarean deliveries). Curettage of the uterine cavity was a routine procedure performed during the cesarean delivery. The maternal characteristics of the groups of pregnant women examined are shown in Table 1. We did not observe any significant differences in the gestational age and features of the newborns between groups.

2.3. Tissue samples

RCAS1 content was estimated in 117 placental tissue samples. In all cases we received the patient's consent. We have also obtained the approval for the research program from the Ethical Committee of Jagiellonian University in Krakow (KBET/379/13/2003).

2.4. Preparation of tissue extracts

Tissue samples (average dimension 0.5 cm × 0.5 cm × 0.5 cm) obtained during the obstetrical procedure were immediately mixed with complete proteinase inhibitor cocktail (Roche, Mannheim, Germany) and homogenized on an ice-bath in a glass/glass Potter-Elvejhem homogenizer. The resulting suspensions were mixed with equal volumes of SDS sample lysis buffer (4% SDS, 20% glycerol, and 125 mM Tris–HCl, pH 6.8) and boiled in a water bath for 5 min. The chilled samples were then spun down at 16,000 × *g* for 15 min at room temperature and the supernatants were used for further analysis.

2.5. Western blotting

Total protein content in the obtained supernatants was measured using BCA assay kit and different sample volumes (usually in the range of 2–10 μl) equivalent to 50 μg of total protein were then loaded onto SDS-PAGE tris-tricine

Table 1
The characteristics of subjects

Pregnants ($n = 117$)	Maternal age ± S.D. (year)	Gestational age ± S.D. (week)	Parity nulliparous (%)	Birth weight ± S.D. (g)	Mean Apgar ± S.D.
Placental abruption ($n = 9$)	28.7 (±3.7)	39.7 (±1.5)	55.5	3354 (±435)	9.42 (±1.45)
Retained placental tissue ($n = 8$)	27.3 (±4.9)	40.6 (±2.21)	62.5	3467 (±208)	9.75 (±0.46)
Spontaneous labor ($n = 40$)	28.5 (±6.1)	39.3 (±1.48)	42.5	3064 (±538)	9.44 (±1.58)
Cesarean elective delivery ($n = 60$)	27 (±4.5)	39.1 (±1.45)	58	3272 (±514)	9.77 (±0.44)

S.D.: standard deviation.