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ORIGINAL ARTICLE

The level of maternal immune tolerance and fetal maturity

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Abstract

OBJECTIVES: Fetal maturity does not seem to be directly connected with the phenomenon of immune tolerance during pregnancy although the fetal maturation influences the process of initiation of the labor at term finishing the immune tolerance during pregnancy. CAP and RCAS1 are expressed by the trophoblast cells and afterwards by the placenta, these proteins are able to modulate the maternal immune response.

MATERIALS AND METHODS: 160 patients were randomly selected to our study. The patients were divided into two groups using K score according to the newborn's maturity: maturated and not fully maturated. Within the groups of matured and not fully matured newborns the subgroups were selected according to the type of the labor: spontaneous or induced. The oxytocinase plasma activity was established in plasma samples obtained from pregnant women a few days before delivery. The placental RCAS1 relative amount was assessed by Western blot analysis.

RESULTS: The differences in oxytocinase plasma level with respect to the fetal maturity were identified in our study however no RCAS1 expression changes were found regarding the fetal maturation. We determined the alterations in RCAS1 expression with respect to the occurrence of clinical symptoms of the spontaneous beginning of the labor in maturated and not-fully maturated groups of newborns.

CONCLUSIONS: Oxytocinase seems to be a useful marker of normal fetal development. The assessment of RCAS1 in placenta directly after delivery appears to indicate the level of maternal immune tolerance during the labor initiation. The level of the immune tolerance at the moment of the delivery drops independently of the fetal maturity.

Introduction

The phenomenon of maternal immune tolerance during pregnancy to fetal antigens explains the possibility of fetal growth within maternal uterus. In 1950-ies Medwar suggested that the suppression of maternal lymphocytes might explain this process [23]. Fetal growth within maternal uterus is much more complex however the suppression of activated lymphocytes is one of its major elements [6,45]. The finishing of this process when the fetus reaches the maturity is the beginning of the labor [14,20]. The cytotoxic immune response grows gradually with the labor

progress [9,43]. The preterm labor which is the labor of immature fetus is similarly to labor at term characterized by changes in maternal immune response activation, but the initiating mechanism is different. The existence of such a complex process as the maternal immune tolerance is determined by the unique mucosa associated lymphoid tissue within endometrium and the role of endometrial cells and trophoblast cells in the process of immune regulation. Both endometrium and endometrium associated lymphoid tissue are controlled by hormones according to menstrual cycle changes [10]. The recruitment of NK, NKT cells and macrophages to the decidua starts during the secretory cycle phase so they constitute almost 95% of all immune cells population within decidua [13]. Mononuclear cells accumulation is assisted by growing number of CD4+CD25high regulatory T cells within decidua, which present ability for immunsupression [11,36,37]. Mainly these cells determine the normal course of pregnancy from ovum implantation until the labor. The activity of immunological system is controlled not only by the decidual cells but also by trophoblast cells through the secretion of various factors eg. cytokins (IL-4, IL-10), hormones (HCG, ACTH), chemokins (CCL22) the expression of various proteins: Fas/FasL (death-inducing signaling complex/CD95/APO-1), indoleamine 2,3 dioxygenase (IDO), killer inhibitory receptor family (KIR), receptor-binding cancer antigen expressed on SiSo cells (RCAS1), oxytocinase/cystine amino peptidase (CAP, EC 3.4.11.3), leukemia inhibitory factor (LIF) [11,12,16,18,25,30,31,35,49]. The decidua, trophoblast and immunological cells all create the phenomenon of maternal immune tolerance to fetal antigens. CAP and RCAS1 are expressed by the trophoblast cells and afterwards by the placenta (syncytiotrophoblast and cytotrophoblast), these proteins are able to modulate the maternal immune response [27,28].

Oxytocinase is responsible for the degradation of oxytocin and other active peptide hormones which are crucial for the normal development of pregnancy [14,17–19,21]. Oxytocinase expression grows gradually during the pregnancy until the labor, the changes in CAP plasma level can be observed in pregnant women as the result of CAP secretion to the maternal plasma. It was shown that the lack of CAP palsma level increase is concomitant to pregnancy loss, preeclampsia and preterm birth [18].

RCAS1 is a protein which was demonstrated in various human cancer cells, responsible for tumor escape from host immunological surveillance however RCAS1 is not only the marker of cancer process, but its expression was also observed in physiological conditions and the development of non-neoplastic tumors [1,27,28,32,40,41,49]. It was demonstrated in bone marrow, endometrium, decidua, placenta, Waldeyer's ring, and immune mediated diseases [1,7,22,48,51]. RCAS1 seems to be responsible for the regulation of the activity of cytotoxic cells.

The aim of the present study was to evaluate the RCAS1 placental expression and CAP plasma level regarding the newborn maturity.

Material and methods

Human subject

We recruited 950 women from the patients delivered at Gynecology and Infertility Department of the Jagiellonian University between March 2002 and March 2004. From this group of patients (950 women) 160 patients were randomly selected to our study. The newborn maturity was evaluated directly after the delivery using K score, pointed by Klimek [20]. The patients were divided into two groups according to the newborns' maturity: maturated and not fully maturated. The first group included 129 women from whom newborns were classified as fully maturated (more than 9 points in K score). The second group consisted of patients from whom newborns were classified as not fully maturated (more than 6 but less than 9 points in K score) (*Table 1*). In both groups patients after vaginal labors (73%) and cesareans (27%) were included. Within the two main groups of matured and not fully matured newborns the subgroups were selected according to the type of the labor: spontaneous or induced. Within the spontaneous labor subgroup women with vaginal delivery of spontaneous onset and regular uterine contractions were included as well as women after cesareans performed during the first stage of labor with ripe cervix and regular uterine contractions. Within the induced labor subgroup women with vaginal delivery induced by oxytocin and cesareans performed with unripe cervix, without regular uterine contractions were included.

Patients with recurrent miscarriage, pervious cesarean section, gestational and pre-gestational diabetes mellitus, previous inappropriate palacentation, precelmapsia and patients with chorioamnionitis were excluded from our study. No patient in our study had taken any hormonal medication during the 6 months before gestation. All obtained tissue samples were histopathologically verified using the classical hematoxylin and eosin staining techniques after fixation in formalin by an experienced pathologist. Patients agreement was obtained in all cases. The approval for the research program of the Jagiellonian University Ethical Committee was also granted (*Table 1*).

Table 1: The characteristics of subjects with respect to the maturity of newborns: not fully and fully maturated.

Pregnant women (n=160) Maturity of newborns	Maternal age ±SD (y)	Gestational age ±SD (wk)	Parity Nulliparous (%)	Newborn length±SD(cm)	Birth weight ±SD(g)	Mean Apgar ±SD
Not fully maturated 6 ≤ K ≤9 (n=31)	29.23(±5.9)	36.19(±2.46)	42	50.4(±2.81)	2729(±586)	8.9(±1.37)
Maturated10 ≤ K ≤12 (n=129)	28.5(±5.07)	38.9(±2.3)	64	52.9(±4.47)	3184(±641)	9.78(±0.6)

Preparation of tissue extracts and Western blotting

The placental RCAS1 relative amount and beta-Actin, which was chosen as a control protein were assessed by Western blot analysis. Relative amount of RCAS1 content was estimated in 160 placental tissue samples taken from normal vaginal deliveries and from cesareans. The used method was described in details in our previous reports [47,49,50]. Briefly, following standard tissue preparation the RCAS1 antigen was identified as a 32kDa band, beta-Actin represented a 42 kDa band [8,44,49].

CAP plasma level

The oxytocinase plasma level was established in 160 plasma samples obtained from pregnant women a few days before delivery. The CAP plasma activity was evaluated using Tuppy and Nesvadba method, modified by Klimek [17]. The assessment of oxytocinase in two pH levels using the same substrate (L-cystine-di-β-naphthylamide) results in obtaining two peeks of aminopeptidase's activity (CAP_1 – pH 7.9; and CAP_2 – pH 6.7). The detailed method of CAP estimation was described in previous studies. The level of CAP_1 in pH 7.9 was considered in our study [14].

Statistical analysis

The distribution of the data was analyzed using Shapiro-Wilk's test. Relative amounts of CAP, RCAS1 and control protein in studied specimens were compared with the use of Student's t-test for normally distributed data and Mann-Whitney U test if non normal distribution was found. Significance of differences between studied groups was set at $p<0.05$.

Results

Statistically significantly lower newborns' birth weight (p=0.006), length (p=0.0008) and gestational age (p=0.00001) were identified in the group of not fully matured newborns in comparison to the group of fully matured newborns (*Table 1*).

Analysis of oxytocinase plasma level

Statistically significantly higher oxytocinase plasma level was identified in patients from whom newborns were classified as fully matured than in patients from whom the newborns were classified as not fully matured (p=0.005). In both examined groups the analysis of oxytocinase plasma activity was performed with respect to the presence of the clinical symptoms of spontaneous beginning of the labor (*Table 2*).

No differences in CAP plasma level were observed between spontaneous and induced labors within the group of not fully matured newborns. Significantly higher CAP serum level was observed in case of induced labor in comparison to spontaneous within the group of matured newborns.

Analysis of placental RCAS1 level

As the amount of β-Actin placental level in all groups was found to be identical (*Table 3*), this indicates that the loading of protein was equal in all samples examined and allows to perform a comparative study between RCAS1 expression between examined groups.

No differences were noticed in RCAS1 expression between matured and not fully matured newborns independently of the type of the beginning of the labor. Statistically significant differences in RCAS1 placental level were identified in two examined groups: not fully matured and matured newborns and regarding the type of the beginning of the labor: spontaneous or induced (respectively $p<0.001$, $p<0.0001$) (*Table 3*).

Within the group of spontaneous delivery no differences in RCAS1 placental level were observed between matured and not fully matured newborns. Similarly no differences were noticed in RCAS1 level in the group of induced labor between matured and not fully matured newborns.

Discussion

The differences in CAP serum level with respect to the fetal maturity were identified in our study however no RCAS1 expression changes were found regarding the fetal maturation. We determined the alterations in RCAS1 expression with respect to the occurrence of

Table 2: Maternal CAP plasma level a few days before the labor with respect to the maturity of newborns: not fully matured and matured.

Variables	Maturity	Not fully matured	Matured
	K-index	6≤ K ≤9(n=31)	10≤ K≤12(n=129)
Average CAP plasma level *μmol/l/min* (±SD)	Spontaneous labor	6.32(±1.12)	7.22(±2.21)
	Induced labor	7.16(±2.11)	9.3(±2.85)

Table 3: Maternal RCAS1 and beta-Actin placental level with respect to the maturity of newborns: not fully maturated and matured.

Variables	Maturity	Not fully matured	Matured
	K-index	6≤ K ≤9(n=31)	10≤ K≤12(n=129)
Relative average of RCAS1 placental amount (±SD)	Spontaneous labor	0,8452(±0,2281)	0,5753(±0,3024)
	Induced labor	1.536(±0.3677)	0.991(±0.3756)
Relative average of beta-Actin placental amount (±SD)	Spontaneous labor	1.1574(±0.6306)	1.0849(±0.7276)
	Induced labor	1.1372(±0.5376)	1.397(±0.4226)

clinical symptoms of the spontaneous beginning of the labor in maturated and not-fully maturated groups of newborns.

Fetal maturity does not seem to be directly connected with the phenomenon of immune tolerance during pregnancy although the fetal maturation influences the process of initiation of the labor at term finishing the immune tolerance during pregnancy. The molecular alterations at the maternal-fetal interface lead to the increasing cytotoxic immune response and are concomitant to the clinical symptoms of spontaneous beginning of the labor and seems to be independent of the gestational age. The miscarriage, preterm delivery, and the labor at term are connected with the growing immune response [2,4,9,24,29,33,43]. The relation between NK cells infiltration of fetal structures and RCAS1 expression drop in trophoblast cells in miscarriage in the third trimester of pregnancy compared with the normal pregnancy was described by Ohshima et al [28]. The participation of NK cells and the growth of Th1 depended response in decidua in miscarriage is well known [35,39]. NK cells are responsible for the control of trophoblast invasion and vascular remodeling in decidua [3]. Normal ovum implantation is also connected with Th1 cytokines secretion, what might indicate that the pregnancy growth is not only dominated by Th2 immunity [39]. Though not only Th1/Th2 balance is responsible for the immune tolerance during pregnancy [5]. CD4+CD25high regulatory lymphocytes seem to participate also in this process [11]. Van Rango et al. demonstrated higher Il-4 concentration in decidua parietalis while INF gamma accumulation in decidua basalis, what indicates different cytotoxic cells distribution around the ovum [46]. Mother recruits NK cells to the decidua during the whole pregnancy [34]. Immune tolerance seems to be the result of dynamic balance between growing cytotoxic response from one side and factors inhibiting the fulfillment of this reaction by the expression of factors and their secretion (Fas-L, KIR, IDO, LIF, RCAS1, CAP and others) at the maternal-fetal interface from the other side [21,25,28,30,31,42]. The shift of the balance to the cytotoxic activity takes place at the labor. The changes in the NK and macrophages number and activity concomitant to the vaginal labor in comparison to the cesarean section were demonstrated by Sindram-Trujillo et al. [38]. There is also a level of immune tolerance even during intrauterine fetal demise and stillbirth which also require the inhibition of immune tolerance and the growth of cytotoxic activity at the maternal-fetal interface, what was confirmed in our previous report when the placental RCAS1 level analysis was performed during stillbirth [50]. Similarly to the analysis of placental RCAS1 expression in preterm delivery higher expression was shown in cases of efficient tocolysis. This might suggest the efficacy of magnesium sulfate therapy perhaps deciding about the level of immune tolerance at the maternal-fetal interface at the moment of its beginning [15]. The growing cytotoxic activity at the maternal-fetal interface and the changes in the uterine cervix determine the labor at term [54]. Both processes are controlled by the immunological system and have to take place in the proper sequence; when the immune response increases too early in decidua basalis placental abruption occurs, when the immune response growth occurs too late the retained placental tissue is observed. It was also described in our previous study, when RCAS1 placental level was considered in cases of retained placental tissue in comparison to placental abruption. Significantly higher RCAS1 expression was identified in cases with retained placental tissue [53]. Recently lower RCAS1 placental expression was observed in cases with the presence of spontaneous beginning of the labor at term in comparison to induced labor at term [49]. These observations were confirmed in the current study in matured newborns, additionally similar difference in RCAS1 placental expression was found in cases of not fully maturated newborns. This might indicate that the level of maternal immune response – immune response inhibition – does not depend on the fetal maturation, whereas the placental RCAS1 level informs about the intensity of the phenomena at the maternal-fetal interface. The analysis of RCAS1 expression with respect to the uterine cervix ripening during cesarean section revealed the statistically significant drop of placental RCAS1 level in cases of cervix dilatation more than 2cm [52].

Significant alterations in CAP plasma level with respect to the beginning of the labor was concomitant only to the labor of matured newborn. This finding is also in compliance with our previous observation concerning the differences in CAP plasma level in a few days before the labor at term according to the type of the beginning of the labor – spontaneous or induced [49]. CAP activity is a good marker of normal pregnancy development [18,26]. In presented study CAP plasma level was raising significantly with the growth of the fetus. Because of correlation between CAP serum activity and the level of fetal maturity CAP is used for the prognosis of the time of delivery [14]. This is why no differences were found between the spontaneous or induced labor of not fully maturated newborn in CAP plasma levels.

In sum our findings might indicate that the evaluation of CAP plasma level and RCAS1 placental expression would inform about the processes taking place at the maternal-fetal interface. CAP seems to be a useful marker of normal fetal development. The assessment of RCAS1 in placenta directly after delivery appears to indicate the level of maternal immune tolerance during the labor initiation. The level of the immune tolerance at the moment of the delivery drops independently of the fetal maturity.

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