Morphology and Function of Placental Macrophages *In Vitro* in Different Outcomes of Pregnancy

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Antigenic phenotype and phagocytic activity of macrophages isolated from the placenta at different terms of gestation and different types of delivery were studied. The expression of MHC II and CR3 antigens and Fc- and C3-mediated phagocytosis increased during gestation. These parameters were increased during labor occurring in the second trimester, which indicates activation of placental macrophages in preterm delivery.

Key Words: *placental macrophages; culture; activation; preterm labor; full-term labor*

Spontaneous abortion is a pressing problem of obstetrics. Efficient treatment of this condition depends on our knowledge of the mechanisms of cell-cell interactions involved in its regulation and realization.

Placenta attracts special interest as an organ maintaining pregnancy; placental macrophages (PM) represent the most numerous population of placental villous stromal cells [2,4], whose role is not confined to participation in nonspecific immune reactions, as was considered traditionally [11]. PM are involved in the initiation and regulation of delivery in both preterm and full-term labor [7,10,17]. Due to its properties and location, this cell population is one of the most probable key components mediating the effects of various stimuli on gestation processes. Activated PM produce bioactive compounds, primarily cytokines modifying the functions of adjacent cells [5, 6,15,17].

We proposed a relatively simple and available procedure for preparing PM culture [1]. In this study we investigated *in vitro* the functional and phenotypical characteristics of cells isolated from the placenta at different terms of gestation after delivery by different methods.

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MATERIALS AND METHODS

Cells were isolated from human placentas (n=30) after spontaneous or induced labor via natural routes or by cesarean section at D. O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, and at St. Petersburg Hospital No. 19. Placentas collected at terms of 21-26 weeks (second trimester), 31-34 weeks (third trimester), and 38-42 weeks (full-term delivery) were divided into 6 groups by the term of gestation and mode of delivery (Table 1). Isolation and culturing of PM has been described in detail [1]. Cells (2×10^5) were placed to 9×9 mm slides and cultured overnight in RPMI-1640 with 15% fetal calf serum (37°C, 5% CO_2).

Cells washed with phosphate buffer (Flow) with 0.2% BSA (PB-BSA, Sigma) were incubated with murine monoclonal antibodies to MHC II antigens (PdV 5.2, anti-HLA D), type 2 Fc receptors (IV.3; anti-FCγRII), and receptors to C3bi complement component (OC-M1; anti-CR3) for 1 h at 37°C (all monoclonal antibodies were a gift from Dr. H. Beekhuizen, University Hospital, Leiden). After 3-fold washing in PB-BSA, FITC-labeled antibodies to murine IgG (Sorbent) were added to the cells for 30 min at room temperature. The cultures were examined under an Opton-Axiophot fluorescent microscope. At least 300 cells per slide were examined. Positively stained cells

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were counted, and the mean values for 3 slides were calculated.

For evaluation of Fc receptor-mediated phagocytosis, sheep erythrocytes (SE) were incubated (30 min, 37°C) with hemolytic rabbit serum diluted 1:200 (Fc-SE). For studies of phagocytosis mediated by receptors to the complement C3 component, Fc-SE were sensitized with C3 by incubating with human AB serum diluted 1:40 (30 min, 37°C) (C3-SE). PM cultures were incubated with Fc-SE or C3-SE for 30 min at 37°C, then washed in PB, non-phagocytized SE were lyzed with 0.83% NH_4Cl, and the cells were stained with Hematoxylin-Eosin. At least 200 cells per slide were examined under microscope; the share of phagocitizing cells (phagocytic index) and number of phagocytosed SE per 200 cells (phagocytic number) were estimated. The mean number of SE phagocytized by one phagocyte and the integral phagocytic index (product of phagocytic index and phagocytic number, divided by 200) were calculated. The mean values for 4 slides were calculated for each placenta.

RESULTS

Cells obtained from the placenta at different terms of gestation without labor (groups 1, 3, and 5) were characterized by different expression of MHC II and CR3 antigens ($p<0.05$). The least number of MHC II-positive cells was observed in group 1 culture. By the middle of the third trimester the number of cells expressing MHC II antigens increased by 30% and slightly decreased by the end of this trimester. Comparison of the expression of MHC II antigens in groups with and without labor activity at the same terms revealed a significant ($p<0.05$) difference only between groups 1 and 2.

The number of cells expressing surface CR3 also increased during pregnancy (group 3 in comparison with group 1) and somewhat decreased by the end of gestation (group 5), although this decrease was statistically insignificant. Expression of FCγRII was equally high in cultures at all terms: virtually all the cells carried this antigen on their surface.

The phagocytic activity of third-trimester cultures (groups 3 and 5) increased in comparison with the second-trimester cultures (group 1, Table 2), Fc-mediated phagocytosis increased by the middle of the third trimester (group 3), while phagocytosis of C3-SE increased only by the end of this period (group 5). Labor notably increased the ability of second-trimester macrophages to phagocytize Fc-SE (group 2, $p<0.05$) and virtually did not modify this parameter in the third trimester (group 4). The phagocytic activity of PM in groups 5 and 6 was virtually the same, but the decrease in Fc- and C3-mediated phagocytosis in group 6 can be regarded as a tendency deserving further investigation.

This study is the first attempt at disclosing a correlation between PM morphology and function in a model system (cell culture) and pregnancy outcome. We detected some regularities in changes of these properties depending on the stage of gestation and type of delivery. In preterm (second trimester) placentas without labor, the phagocytic activity of PM was the lowest. This correlated with immunocytochemical parameters: low expression of CR3 receptors in comparison with later terms. However, the number of CR3-positive cells increased in the middle of the third trimester, while the parameters of C3-mediated phagocytosis increased only at the end of gestation. The high level of FCγRII expression in cells of all terms can be explained by the necessity of binding immune complexes at the early stages of development for providing fetal defense against alloimmune response [13].

Increased expression of MHC II antigens from the second to the third trimester is in line with the data of immunohistochemical analysis of placental tissue, demonstrating that the number of MNC II-positive cells

TABLE 1. Antigenic Phenotype of PM *In Vitro (M±m)*

Groups	Delivery	Number of positively stained cells, %		
		MHC II	FCγRII	CR3
Trimester II				
1	No labor	61±14	99±2	58±22
2	With labor	87±9	—	—
Trimester III				
3	No labor	92±3	99±1	96±3
4	With labor	91±11	—	—
Full-term labor				
5	No labor	81±7	99±1	80±12
6	With labor	90±9	—	—

TABLE 2. Phagocytosis of SE in PM Culture ($M \pm m$)

Groups	Delivery	Fc-mediated phagocytosis			C3-mediated phagocytosis		
		IPI	% of phagocytes	PN_1	IPI	% of phagocytes	PN_1
Trimester II							
1	No labor	0.47±0.21	43±14	2.2±0.2	0.55±0.26	46±13	2.4±0.4
2	With labor	1.0±0.17	68±11	2.2±0.2	—	—	—
Trimester III							
3	No labor	0.81±0.25	60±14	2.1±0.3	0.43±0.23	48±18	1.7±0.3
4	With labor	0.7±0.22	61±10	2.0±0.2	—	—	—
Full-term labor							
5	No labor	0.81±0.24	57±11	2.2±0.2	0.93±0.22	63±7	2.2±0.3
6	With labor	0.67±0.26	52±13	1.6±0.3	0.75±0.35	62±16	1.7±0.2

Note. IPI: integral phagocytic index; PN_1: mean number of sheep erythrocytes phagocytized by one phagocyte.

increased with age [4,8]. High expression of MHC II and CR3 and intense phagocytosis suggest an increase in the functional activity of immunocompetent cells [3]. Our experiments demonstrated a 20-30% increase in functionally active PM from the middle of the second to the second half of the third trimester. Therefore, the function of PM population as the regulatory-effector component of the fetoplacental immune system becomes more efficient with age.

Hypotheses on a possible role of PM in preterm and full-term delivery [7,10,17] make us regard this cell population as an important element in gestation processes. In this connection activation of PM can be considered as an event associated with preterm or full-term delivery [7,10,14]. Recent studies revealed a positive correlation between high levels of some cytokines (tumor necrosis factor-α, interleukin-1, and interleukin-6) produced by activated macrophages and the onset of preterm or full-term labor [9,12,14,16]. We investigated the relationship between labor activity at different terms of pregnancy and the degree of PM activation *in vitro,* which was evaluated by the level of MHC II antigen expression and phagocytic activity of cells mediated by Fc and C3 receptors.

Based on published reports, we suggested that the level of macrophage activation increases during labor. However, the majority of activation parameters notably increased in the second trimester cell cultures in case of labor (Tables 1 and 2, groups 1 and 2). The number of cells expressing MHC II and CR3 and capable of phagocytosis increased. At later terms labor was not accompanied by activation of PM (groups 3 and 6). Our findings suggest that the level of phagocytosis in group 6 decreased due to low activity of individual cells, while the number of phagocytes remained practically unchanged. In order to elucidate whether this tendency reflects a regularity or results from heterogeneity of the studied material, more placentas from each group should be examined.

The results indicate good prospects of using PM-enriched cultures in studies of immunological reproduction, specifically, for disclosing the cellular mechanisms underlying normal and preterm labor.

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