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Localization of Activation Marker CD_{27}on Peripheral Blood Lymphocytes and Placenta of Rats with Prenatal Hypoxia

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Abstract: Factors contributing to the development of fetal hypoxia are very numerous. Degree of immune-hormonal disorders depending on the severity of prenatal hypoxia has not been studied in detail. In this study we investigated the localization of the activation marker CD_{27+} peripheral blood lymphocytes and placenta of rats with prenatal hypoxia of varying severity. Study the localization CD_{27+}markers revealed disorders of immune cells to the blood and placenta of rats during hypoxia of varying severity. Changes in CD cell differentiation leads to functional immature T-lymphocytes. The study revealed violations of functional immune parameters on systemic and local levels contribute to the pathological course of pregnancy and may result in unfavorable outcome.

Key words: Prenatal Hypoxia · Blood · Placenta · CD-Lymphocytes

INTRODUCTION

The state of intrauterine fetal hypoxia is important demographic problem today. In the structure of perinatal mortality hypoxic condition occupy one of leading places (from 5.1% to 12.8%). Children, who had perinatal hypoxia, have neurological disorders, respiratory syndrome disorders, dysfunction of gastrointestinal tract [1]. The state of immune homeostasis mother, formed under the influence of pathological factors in complicated pregnancy, determines the type of immune response newborn during early postnatal adaptation [2].

Hypoxia is a powerful stress factor, which is supposed to lead to a rise not only of erythrocytes with compensatory purpose, but the number of other blood cells as a result of cytokine mobilization in response to stress.A number of studies have shown that severe hypoxia in vitro promotes self-renewal of mouse and human HSCs [3]. Response to hypoxia was also a strong division of CD_{34+} cord blood cells [4].

In recent years, much attention is paid to the elucidation of molecular mechanisms that regulate the activity and migration of lymphoid cells, including receptor CD_{27} role in the differentiation of effector T-lymphocytes, the expression level of which varies during development and maturation of the cells [5]. During pregnancy hypoxia is poorly understood. Given the above, the localization of molecules was investigated CD_{27} during differentiation of T-lymphocyte subpopulations CD_{3+}, CD_{4+}, CD_{8+} and CD_{16+} lymphocytes in peripheral blood and the placenta in rats during pregnancy complicated by hypoxia of varying severity.

MATERIALS AND METHODS

The experiment was conducted on white rats females, weighing 225-230 g, which created varying degrees of hypoxia (I, II, III degree)during pregnancy. The control group consisted of healthy rats with physiological pregnancy. Peripheral blood sampling was performed after giving light ether anesthesia by decapitation in sterile disposable tubes with EDTA anticoagulant. Lymphocytes were isolated from rat placenta one gram homogenized placental tissue. Identification of lymphocytes was carried out based on the total leukocyte the gate CD_{45}.

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Study on localization CD_{27} molecule CD_{3+}, CD_{4+}, CD_{8+}, CD_{16+} lymphocytes were performed by direct immunofluorescence membrane flow cytometer BD Facs Calibur [6] using a panel of monoclonal antibodies to lymphocyte surface antigens using double phenotyping.

Using commercial reagent kits fits labeled anti-rat CD_{3+}, CD_{4+}, CD_{8+}, CD_{16+} and PE anti-rat CD_{27} (BD Biosciences, USA). All data obtained were subjected to statistical analysis with the use of Student criterion.

RESULTS AND DISCUSSION

Study localization activation marker CD_{27} peripheral blood lymphocytes in rats during pregnancy complicated by hypoxia are shown in Table 1. Data analysis revealed that hypoxia in the peripheral blood of pregnant rats showed an increase in the number of cells bearing the receptor CD_{27+}, compared with those obtained with physiological pregnancy.

This applied mature CD_{3+} lymphocytes (II, III degree of hypoxia), helper - inductor CD_{4+} cells (I, II, III degree of hypoxia) ($P<0.05$). When this buildup CD_{27+} marker expression on cells corresponded severity of hypoxia in rats (CD_{3+} and CD_{4+} cells). CD_{27+} receptor expression on the suppressor-cytotoxic CD_{8+} T cells during hypoxia I and II did not differ from similar indicators identified in physiological pregnancy ($P>0.05$), but was decreased in the III degree of hypoxia ($P<0.05$). Of natural killer cell phenotype CD_{16+} CD_{27+} receptor localization in comparison with the data of physiological pregnancy significantly increased when I degree of hypoxia and then significantly decreased as the severity of hypoxia (II, III degree). CD_{27-} receptor localization on CD $_{3+}$lymphocytes of rats during hypoxia I, II, III severity was significantly lower compared with those obtained with normal pregnancy ($P<0.05$) and decreased depending on the severity ofhypoxia ($P<0.05$). This indicates a decrease in the functional activity of mature CD_{3+} cells carrying cellular immune responses during hypoxia. CD_{27-} receptor localization on-helper CD_{4+} cells inductor during hypoxia I and II was significantly larger than in normal pregnancy, but decreased with the III degree of hypoxia ($P<0.05$), describing the severity of hypoxia and functional immaturity of CD_{4+} cells carrying the protective immunity. Among suppressor - cytotoxic CD_{8+} T-lymphocytes in peripheral blood of rats CD $_{27-}$ receptor expression during all stages of hypoxia was significantly lower than in normal pregnancy and decreased as the severity of hypoxia ($P<0.05$). This indicates a functional immaturity suppressor-cytotoxic T-cells exhibiting a cytotoxic effect. Of natural killer cell phenotype CD_{16+} CD_{27-} receptor

Table 1: Localization of activation marker CD_{27} peripheral blood lymphocytes of rats during hypoxia of varying severity

		The degree of severity hypoxia		
Name of indicators	Physiological pregnancy	I	II	III
CD_{3+}/CD_{27-}	26.54±2.15	16.7±1.03[a]	[b]4.17±0.24[a]	[b]1.49±0.11[c]
CD_{3+}/CD_{27+}	51.82±1.07	52.87±2.06	[b]57.72±1.27[a]	[b]63.25±1.23[c]
CD_{4+}/CD_{27-}	2.05±0.09	3.86±0.25[a]	[b]3.04±0.23[a]	[b]2.60±0.11[a]
CD_{4+}/CD_{27+}	11.51±0.28	31.80±1.87[a]	[b]41.72±1.81[a]	[b]46.75±1.02[c]
CD_{8+}/CD_{27-}	30.88±1.35	18.06±0.50[a]	[b]2.19±0.11[a]	[b]0.47±0.07[c]
CD_{8+}/CD_{27+}	20.89±2.21	19.93±0.31	22.43±0.96	[b]11.32±0.63[c]
CD_{16+}/CD_{27-}	0.67±0.06	0.72±0.06	0.71±0.06	[b]0.24±0.02[c]
CD_{16+}/CD_{27+}	0.79±0.11	1.79±0.11[a]	[b]0.71±0.03	[b]0.12±0.01[c]

Note:[a]P<0.05- Physiological differences between pregnancy and hypoxia; [b]P<0.05-differences between the I, II, III degree of hypoxia; [c]P<0.05-the difference between the II and III degree of hypoxia.

Table 2: Localization CD_{27} activation marker on lymphocytes of the placenta in rats during hypoxia of varying severity

		Hypoxia (Degree)		
Name of indicators	Physiological pregnancy	I	II	III
CD_{3+}/CD_{27-}	20.67±0.37	31.12±1.44[a]	[b]8.58±0.21[a]	[c]21.19±0.55[b]
CD_{3+}/CD_{27+}	0.46±0.07	14.53±0.35[a]	[b]7.53±0.24[a]	[b]1.94±[c]0.11[a]
CD_{4+}/CD_{27-}	23.15±0.10	4.33±0.18[a]	0.97[b]±0.04[a]	[b]2.78±[c]0.09[a]
CD_{4+}/CD_{27+}	0.37±0.02	[a]2.20±0.10	[a]2.62[b]±0.12	[a]1.13[b]±0.06[c]
CD_{8+}/CD_{27-}	15.62±0.11	[a]12.17±0.09	[a]3.38[b]±0.07	[b]11.17[c]±0.35[a]
CD_{8+}/CD_{27+}	2.25±0.22	[a]0.80±0.01	[a]0.79±0.02	[a]0.86[b]±0.01[c]
CD_{16+}/CD_{27-}	1.04±0.21	0.50±0.01[a]	1.88[b]±0.06[a]	[b]0.56[c]±0.02[a]
CD_{16+}/CD_{27+}	0.80±0.01	0.30±0.02[a]	[b]0.24±0.01[a]	[b]0.12[c]±0.008[a]

Note:[a]Ð<0.05 -the distinction between physiological pregnancy and hypoxia; [b]Ð<0.05- the differences between the I, II, III degree of hypoxia; [c]Ð<0.05- the differences between the II and III degree of hypoxia.

localization in I and II degrees of hypoxia did not differ from values obtained in normal pregnancy (P>0.05), but significantly decreased at the III degree of hypoxia (P<0.05). The data show that hypoxia is accompanied by severe functional immaturity of natural killer cells CD_{16+} phenotype.

It is known that the favorable outcome of pregnancy largely determine immunity factors involved in the interaction of maternal cells and trophoblast in decidua i.e. in contact maternal tissue and fetal [7], etc. In this connection investigated activation marker localization CD_{27} on a local level, *i.e*. placental hypoxia rats was illustrated in Table 2.

Analyzing the results of Table 2, showed that during hypoxia in the placenta in rats, an increase in the localization of the receptor CD_{27+} mature CD $_{3+}$(I-III), helper-inductor CD_{4+} (I-III degree) T-lymphocytes compared to the data for normal pregnancy (P<0.05). Conversely, on the suppressor-cytotoxic CD_{8+} T cells of the placenta during hypoxia in rats was recorded decrease receptor localization CD_{27+} to CD_{8+} lymphocytes (I, II, III degree) P<0.05. Such a decrease in receptor localization SD_{27+} observed at hypoxia and natural killer cells CD_{16+} phenotype (I, II, III degree) (P<0.05) *i.e*. on separate subpopulations of lymphocytes placenta of rats during hypoxia, in contrast to the data in physiological pregnancy, increased receptor localization CD_{27+} tested on mature CD_{3+} and immunoregulatory CD_4 helper-inductor + lymphocytes placenta at all degrees of hypoxia. On immunoregulatory suppressor - cytotoxic CD_{8+} T cells and natural killer cells of the placenta CD_{16+} rats, on the contrary, with all degrees of hypoxia and in contrast to the norm, tested decrease localization CD_{27+} receptors. CD_{27} marker of individual CD $_{3}$, CD $_{4}$, CD $_{8+}$ CD_{16+} lymphocytes placenta of rats can regard these shifts as a violation of cell differentiation during hypoxia.

Depending on the severity of hypoxia localizing molecules increase of CD_{27+} CD_{3+} lymphocytes placenta was the highest when the degree of hypoxia I, unlike II and III (P<0.05) and significantly decreased then, taking the lowest value when the degree of hypoxia III (R< 0.05). Among the CD_{4+} cell increase of the marker was CD_{27+} II at the highest power, unlike I and III (P< 0.05) and the lowest degree at III in contrast to I (P<0.05). Of suppressor-cytotoxic CD_{8+} T lymphocytes decrease placental receptor localization CD_{27+} depending on the severity of hypoxia noted in the I and II compared to III (P<0.05). Among CD_{16+} natural killer cell receptor localization reduction CD_{27+} is at least increasing severity of hypoxia and was the lowest when the degree III unlike II and I and II-I, in contrast to the degree (P<0.05). CD_{27-} receptor expression on CD_{3+} lymphocytes placenta of rats during hypoxia in contrast to similar indicators registered in physiological pregnancy revealed an increase in receptor localization of I degree of hypoxia and then a significant decrease in the II degree and normalize the data at III degree hypoxia (P<0.05).

Localization CD_{27-} on CD_{4+} lymphocytes placenta of rats during hypoxia in general and on the severity of hypoxia was decreased in I, II, III degree (p<0.05) in contrast to similar indicators norm, but the lowest in the II degree. CD_{27-} localization of molecules on the suppressor-cytotoxic CD_{8+} cells of the placenta in rats during hypoxia was significantly decreased in each degree of hypoxia, unlike similar physiological indicators of pregnancy (P<0.05), but the lowest at II degree. CD_{27-} receptor localization on natural killer cells CD_{16+} phenotype rats during hypoxia was lower physiological values of the norm when I and III degrees of hypoxia and up at IIdegree hypoxia (P<0.05). In the dynamics of hypoxia were highest localization values CD_{27-} CD_{16+} lymphocytes by the placenta when I degree of hypoxia and lowest with the III degree of hypoxia (P<0.05). Down regulation CD_{27} receptor on CD_{8+} cells of the placenta at all degrees of hypoxia, as well as lymphocytes of CD_{16+} I and III, indicating functional immaturity as a suppressor-cytotoxic or placental natural killer cells and killer carrying cytotoxic effect. An increase in the expression of the receptor on the CD_{27-} CD_{16+} killer cells in the II degree of hypoxia compared with normal values (P<0.05), it means temporary increase natural killer cell function, which fade as the severity of hypoxia.

The immune system in prenatal hypoxia is a leading criterion usefulness homeostatic mechanism of reproductive processes [8]. It is known that lymphocyte phenotypic testing indicators in dynamics provides additional information about the current state of the body and can be used to monitor the status of the immune system. Physiological immaturity of the immune system of the fetus / newborn cannot provide a sufficient level of effective protection of anti-antenatal and early postnatal period [9]. It was shown that functional classification reflects the phenotypic classification of CD_{8+} T cells [10].

When pregnancy is observed changes in the subpopulation composition. In the first two trimesters of pregnancy, the decrease of the absolute number of T-helper cells, while the relative number is not changed. This index is normalized to the third trimester [11]. In the

early stages normal pregnancy note the increase in the relative number of T-regulatory (Treg) in peripheral blood than non-pregnant women [12].

In recent years, much attention is paid to the elucidation of molecular mechanisms that regulate the activity and migration of lymphoid cells, including CD27 receptor role in the differentiation of effector T-lymphocytes, the expression level of which varies during development and maturation of the cells [5]. When prenatal hypoxia this question actually has not been studied. It has been shown that chronic fetal hypoxia leads to a decrease in the amount of CD_{34+} cells cloning efficiency and increase the level of spontaneous apoptosis of leukocytes. In acute hypoxia number of CD_{34+} cells, red blood cells, hemoglobin concentration statistically significantly higher viability of leukocytes and hematopoietic stem cells of umbilical cord blood does not change [13, 14]. Change in lymphocyte subpopulations of peripheral blood CD_4 fluctuation levels and CD_{8-} lymphocytes leads to a decrease in the immunoregulatory index, which is one of the main indicators characterizing the harmonious functioning of the immune system.

It is known that effector CD_{27+} lymphocytes consist of 2 subpopulations: CD_{27+} CD_{27-}. At long antigenic stimulation CD_{27+} lymphocytes differentiate into cells $CD_{27-}$and lymphocytes CD $_{27}$do not restore the expression of molecules CD_{27} [15]. The loss of cells in the expression of receptors CD_{27} closely associated with the acquisition of the cell functional activity. It was concluded that the lack of surface expression of molecules CD_{27} is a characteristic functional of mature T-cell and differentiation CD_{27+} CD_{27} is a stage in which a cell becomes functional activity.

It was found that in healthy women in the peripheral blood of expression CD_{27} receptor on CD_{4+} immunoregulatory cells prevails over the expression of CD_{27+} molecules. It is an attribute of presence of sufficient number of functionally mature effector CD_{4+} at the system level. Inflammatory diseases forms immuno deficiency among CD_{4+} helper/induction of T-lymphocytes, the number of which is lower than in the group of healthy women,which increased after treatment siliceous mineral water. Functional immaturity of CD_{4+}, apparently, is one of the causes of violation of immune regulation in women and contributes to the disturbances in the reproductive system [16-18].

In our experiments hypoxia causes serious changes in the immune system, violating the differentiation of T-lymphocytes in the peripheral blood, which leads to their functional immaturity and contributes to the development of pathology of pregnancy. It is tested by a decrease in the expression of molecules CD_{27} mature CD_{3+} (I, II, III degree of hypoxia), CD_{4+} (III) the degree of hypoxia), CD_{8+} (I, II, III degree of hypoxia), CD_{16+} (III) the degree of hypoxia) lymphocytes.

CONCLUSION

Prenatal hypoxia causes disturbance localization CD_{27+}, CD_{25+} receptors on immune cells of peripheral blood and the placenta in rats with prenatal hypoxia, which depends on the severity of hypoxia.

Study localization CD_{27} marker on immunocompetent cells of the placenta in rats during hypoxia of varying severity revealed changes differentiation CD_{3+}, CD_{4+}, CD_{8+}, CD_{16+} placental cells, differ from that obtained in normal pregnancy, which led to functional immaturity of T-lymphocytes CD_{3+} phenotype (II degree), immunoregulatory CD_{4+} (I-III degree), CD_{8+} (I-III degree) phenotypes, natural killer cells CD_{16+} phenotype (I and III). The identified violations of the functional parameters of immunity system and local levels contribute to the pathological course of pregnancy and may lead to its unfavorable outcome.

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