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Further Characteristics of Pregnant Bone Marrow Cells Action on Rat Development during Early Pregnancy

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Abstract

There were early results that effect of single intravenous transplantation of pregnant rats Percoll derived mononuclear bone marrow cells (BMC) suspention of 4-5, 7-9 or 11-12 pregnant days to rats with the same date of pregnancy depends on gestation period of transplantation. The weight of 18th day fetuses after intravenous transplantation during 7-9 days was increased with significant difference in comparison with weight of normal fetuses. In case of intravenous BMC transplantation during placentation at 11-12 days of pregnancy the weight of fetuses was decreased, the weight of placentas was increased and survival of fetuses was disturbed. There are several explanations of increase of weight of fetuses after intravenous BMC transplantation within gastrulation including paracrine effect of allogenic transplanted cells on the DC development, the apoptosis and proliferation of embryonal cells and possible epigenetic action. The retardation of fetuses growth after intravenous BMC transplantation during placenta formation at pregnant 11-12 days may be explained by cytotoxic action of uNK cells for embryo. At the same time the sub-dermal BMC transplantation at 11 and 13 pregnant days stopped the embryo-toxic action of uNK cells and increased the weight of fetuses and preserved the survival of embryos.

Keywords Pregnancy; Bone marrow; Transplantation

Introduction

Cell interactions between the uterus and the fetus determine the success or failure of pregnancy and fetal development in mammals. Cellular and molecular interactions are particularly active in species with interstitial implantation and hemo-chorial type of placenta (human, rodents, some monkeys, bats and insectivores). In this case fetuses were surrounded by the decidual cells (DC), which differentiated around the fetus in the endometrium, forming the mother part of the placenta and the peripheral part of the decidual membrane.

Early we estimated that intravenous transplantation of bone marrow cells (BMC) of pregnant rats to other pregnant rats during gastrulation increased the weight of the fetus of 18 day of pregnancy. At the same time pregnant BMC transplantation during placentation (11-12 days of pregnancy) decreased the weight of fetuses and increased of death level [1].

Blastocyst development of experimental animals both *in vivo* and *in vitro* without DC stops at the initial stages of gastrulation and does not go beyond somite formation [2,3]. DC participate in the trophic relationships between mother and fetus and hormonal regulation of pregnancy, their specific functions include the prevention of the development of inflammation in the endometrium and the regulation of immunological conflict between mother and fetus [4]. In accord with modern results a human embryos influence embryo implantation and functional state of DC depending on the state of embryos [5].

Relationships between pregnant endometrial cells, DC formation, pregnancy and embryogenesis are of great interest. Allogenic transplantation of rat lymphocytes in the uterus of pregnant rats on days 12-20 of pregnancy was not associated with the damage of the fetus tissues [6]. Intraperitoneal allogenic transplantation of BMC of mice on day 13 of pregnancy resulted in the long-term, up to 2 weeks, survival of transplanted cells in recipients [7]. According to our data, transplantation of BMC derived from non-pregnant female rats to rats endometrium at 5th day of pseudopregnancy enhances the growth of DC [8]. There is also resulting that mice bone marrow is characterized by a maximum of nuclear cell concentrations on days 6-9 of pregnancy [9].

This paper describes the comparative effect of intravenous and sub-dermal transplantation in pregnant rats of BMC, extracted from pregnant rats of the same gestational age, on the growth of the fetuses at 18th day of pregnancy. The transplantation was carried out on 4-5 days of the pre-implantation period of pregnancy and 7-13 days of post-implantation period of pregnancy. Since it is assumed that BMC transplantation affects both DC and embryogenesis, the results of transplantation were evaluated on day 18 of pregnancy, as it was made early when we studied teratogenicity of anti-kidney immune serum after intra-peritoneum or intravenous injections on 8-10 days of pregnancy [4,10]. The presented results partly repeats the previous results at more quantitative level [1] and support point of view that rat embryo during post implantation period of development can be regulated [11]. Pregnant BMC mother cells may be as regulator.

Materials and Methods

The outbred white rats, weighting 200 to 250 g, were obtained from specialized animal farm "Rappolovo" (St. Petersburg, Russia). Rats were maintained on standard laboratory the 12 h light/dark cycle. All procedures performed on rats were carried out according to the guidelines for humane care of laboratory animals. The day of observation of spermatozoid in the morning vaginal smear was

marked as the first day of pregnancy [10]. Bone marrow cells (BMC) were prepared from long bones of pregnant rat. After 63% Percoll fractionation mononuclear cells were collected and injected intravenously (*V. jugularis*) in volume 1 ml PBS to rat of the same date of pregnancy. In case of subdermal transplantation cells were injected in 10 points of abdominal part of body in volume 0.1 ml. The quantity of mononuclear cells for every transplantation was (135 ± 12) 106. As control we used normal fetuses of 18th day of pregnancy and transplantation of BMC from diestrus rats to 7th and 8th day pregnant rats. Injections were made under diethyl ether narcosis. The results were counted from 96 pregnant rats. The animals were opened at 18th of pregnancy with the help of diethyl ether narcosis. The fetuses and placentas were fixed at Bouin solution 24 h and weighted the next day. Action of Bouin solution is characterized by high speed and by absence of tissue damage without change of fetus body volume and weight [10]. First of all we weighted fetuses and placentas of every family and counted mean value for every family. The results of weight of fetuses and placentas of every family were combined to make experimental groups. The first point for comparison data was group of normal fetuses of 18th day of pregnancy because it was the same for all experimental groups. The mean values of groups were compared with each other. The level of preimplantation death was counted as discrepancy of quantity of yellow bodies in ovary and quantity of implantation sites at uterus; the level of post implantation death was counted as discrepancy between quantity of implantation sites and quantity of live fetuses. Statistical analyses were made in accord with recommendations of Lang et al. [12]. All groups data are given as mean ± SD. Statistical significance between groups was calculated in accord with Student's t-test.

Results

Aspirated bone marrow was further processed in order to isolate the mononuclear fraction, a heterogeneous cell population containing differentially matured B-cells, T-cells and monocytes, as rare progenitor cells such as hematopoietic stem cells, mesenchymal stromal cells, endothelial progenitor cells and very small embryonic-like cells. In accordance with multiple studies such cell mixture promotes distinct angiogenic properties, mediates vascular repair, express several cytoprotective growth factors and cytokines [13]. Practically BMC intravenous transplantation dramatically increases level of blood BMC concentration up to concentration which can't be reached by another experimental procedure. It was shown that transplantation of BMC of pregnant rats into pregnant rats of the same date of pregnancy is not accompanied by teratogenic effects in accord after superficial examination of embryos.

BMC transplantation before implantation at 4-5 days of pregnancy was characterized by an increased level of pre- and post-implantation death of embryos with significant difference to normal rats , $P<0.05$ (Table 1). The post-implantation survival of fetuses had tendency to be decreased after intravenous transplantation of pregnant BMC at 11-12 days of pregnancy.

Pregnant days of BM cells transplantation	Pre-implantation embryos survival, %	Post-implantation embryos survival, %
Normal foetuses of 18th day pregnancy (28 rats)	94.2 ± 2.4	94.8 ± 2.7
BMC intravenous transplantation at 4-5 days of pregnancy (4 rats)	72.3 ± 8.5	45.0 ± 15.4
BMC intravenous transplantation at 7-9 days of pregnancy (15 rats)	96.6 ± 2.3	98.2 ± 1.3
BMC intravenous transplantation at 11-12 days of pregnancy (3 rats)	89.3 ± 6.7	71.7 ± 11.8
BMC subdermal transplantation at 11 day of pregnancy (8 rats)	91.7 ± 5.6	89.3 ± 5.1
Diestrus rats BMC (control) intravenous transplantation during pregnant 7-8 days (4 rats)	92.6 ± 15	87.8 ± 16

Table 1: Pre- and post-implantation survival of rat's fetuses of 18th day of pregnancy after transplantation of mononuclear bone marrow cells (BMC) during early stages of pregnancy (%).

The high level of pre- and post-implantation death may be explained by the disturbance of implantation due to the excesses of transplanted BMC. Probably an excess of BMC violates the coordination of numerous molecular and cellular mechanisms which regulate the interaction of blastocysts and uterine during implantation [14]. The weight of fetuses at 18th day of pregnancy does not differ from the normal and control pregnant rats that can be explained by the absence of the pathological influence of BMC on blastocysts development after BMC transplantation.

We observed an increased weight of 18th day fetuses in case of BMC intravenous transplantation during gastrulation at 7-9 days of pregnancy (Table 2). Intravenously transplantation of BMC during placentation at 11-12 days of pregnancy induces the retardation of fetuses growth (Table 1). Retardation of growth may be explained by appearance in BM of granulated endometrial cells (EGC) with NK activity [15-18]. According to Podporina EGC are characterized by uNK activity at the beginning and by immuno-suppressor activity at the terminal stage of their differentiation. These capacities of granulated cell activity may explain the function of rat granulated cells as regulators of immunological interaction in placenta [19]. Granulated cells of endometrium (EGC), being the part of DC, are characterized by natural killer activity. According to our data rat EGC produce cell specific antigen with the non-specific esterase activity [20] and participate in the regulation of relationship between mother and fetus. The EGC are also characterized by secretory activity [21].

Time of BMCs transplantation	Weight of 18th day fetuses (mg)	Weight of 18th day placenta (mg)
Normal fetuses of 18th day of pregnancy (28 rats)	745 ± 11	325 ± 8
Intravenous BMC transplantation at 4-5th day of pregnancy (4 rats)	760 ± 20	-
Intravenous diestrus (control) BMC transplantation at 7-8th day of pregnancy (4 rats)	772 ± 9)	343 ± 12
Intravenous transplantation of BMC at 7-9th days of pregnancy (15 rats)	835 ± 15	317 ± 17
Intravenous transplantation of BMC 11-12th days of pregnancy, (3 rats)	587 ± 5	375 ± 50
Subdermal transplantation of BMC of 11th and 13th day of pregnancy, (8 rats)	861 ± 28	314 ± 15

Table 2: Weight of rat fetuses of 18th day of pregnancy after bone marrow cells transplantation at post-implantation stage of pregnancy.

To control the embryo retardation after intravenous transplantation of BMC we made BMC transplantation by sub-dermal injections during 11 and 13 pregnant days. At 18th day of pregnancy the weight of fetuses was increased and weight of placentas was at control level (Table 2). We suggest that sub-dermal transplantation BMC do not allow uNK to reach embryos quickly and to damage it by direct contact. At the same time the preservation of EGC in the sites of sub-dermal BMC transplantation continue to influence the survival of embryos by its secretory activity. The absence of placentas weight changes may be also interpreted as pointing to BMC influence on fetal organogenesis through yolk sac. In every case the increased embryo weight with high level of fetuses survival (Table 2) and absence of signs of teratogenicity shows for general positive effect of BMC transplantation for fetuses development during gastrulation and organogenesis. Comparison of confidence interval (CI) of average weight of fetuses with value at 95% level show that CI of fetuses after BMC intravenous transplantation at 7-9 days (CI: 805–864) and at 11-13 days of pregnancy after sub-dermal transplantation (CI: 803–919) exceed the CI of normal fetuses of 18th day of pregnancy (CI: 723–766) [12]. In means that mean weights of fetuses significantly shift to the larger level after transplantation of BMC by sub-dermal method of transplantation. The change of weight of placentas had not so dramatic character.

Discussion

It is known that mammalian growth is regulated by somatotropin or by Growth Hormone (GH). GH also regulates balanced growth of body. Absence of GH induces nanism without disturbance of balanced growth of body. After transplantation of mononuclear pregnant BMC the fetuses also characterized by proportionality of bodies [1]. At that time the level of post-implantation fetuses death do not grow up.

The increase of fetuses weight after BMC transplantation during post-implantation stage of development may be explained by positive paracrine effects of transplanted allogenic BMC or/and to DC or embryo cells. The strengthening of weight of fetuses at 18th day of pregnancy after BMC transplantation during gastrulation and organogenesis may be also explained by the multiplication of cells participated during gastrulation and organogenesis. Gastrulation includes the formation of mesoderm and endoderm, determination of body axes and of size of organ rudiments [11,18,22]. Gastrulation is regulated by sets of morphogenetic signals and of transcriptional factors [23]. Rat gastrula is characterized by the high level of cells proliferation. In accord with autoradiographical results peculiarity of gastrula cells proliferation belongs to the maximum shortening of cell cycle data and to significant labeling index, approximately 62%. Primitive steak has very high labelling index-about 72%. It means that transplanted pregnant BMC can't increase the proliferation rate of gastrula cells in a greater degree. At the same time Auley's flow cytometrical results points to the physical presence of cells in the state of apoptosis during 8.5-9.5 days of rat gestation [24].

Programmed cell death is an obligate event of morphogenesis and organogenesis [25]. The mechanism of teratogenesis can be described as a negative local balance of embryo morphogenetic cell death and cell proliferation after teratogen action. There are not examples of teratogenesis with increased growth of fetuses [26]. Participation of apoptosis in morphogenesis is demonstrated by differentiating cell systems like neurogenesis [27]. There is possibility that BMC transplantation during gastrula formation and organogenesis retards cell apoptosis level that in turn allow increasing the growth of fetuses.

The absence of results for survival of BMC after transplantation does not allow determining the life longevity of transplanted cells. It is very difficult to explain the significant growth of fetuses at 18 pregnant days by the increase of quantity of embryo cells during gastrulation. Must be another mechanism to explain increased growth of fetuses weight up to 18 day of pregnancy. May be the epigenetic reprogramming during gastrulation could be reason of increased fetuses growth during next days of development. Placental mammals such as rodents and humans are characterized by epigenetic reprogramming or genomic imprinting. The main manifestation of genomic imprinting is the necessity of the paternal genome for fetal development [28]. There is appearance of results for participation of epigenetic reprogramming in rodent gastrulation [29-31]. There is a large interest to explain the growth effect of stem cells transplantation by epigenetic reprogramming during gastrulation.

Common results show that increase of allogenous BMC concentration in the blood of pregnant rat safter intravenous transplantation is characterize by specific features embryo development at every stage of pregnancy. In case of pre-implantation period we observed the increase of embryo loss without decrease of weight of fetuses at 18th day of pregnancy. During gastrulation there is enlargement of weight of fetuses and absence of decrease of weight of fetuses and placentas. During placentation (11-12 days of pregnancy) there is decrease of weight of fetuses and preservation of weight of placentas. The use of sub-dermal transplantation of BMC during placentation increases the weight of fetuses and stabilizes the weight of placentas. The change of weight of placentas was not so strong.

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