Role of Placenta in the Regulation of Fetal Erythropoiesis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 11, pp. 519-521, November, 2001
Original article submitted August 14, 2001

We studied erythropoiesis in newborn infants delivered by mothers with normal pregnancy and gestosis. The effects of placental extracts on hemopoiesis in JCR mice were also evaluated. Our results suggest that the placenta is involved in the regulation of fetal erythropoiesis. The placenta activates fetal erythropoiesis during physiological pregnancy, while in pregnant women with gestosis the erythropoiesis-stimulating effect of placentas was less pronounced, which probably determines low reticulocyte content in the umbilical blood.

Key Words: *placenta; fetus; newborn; erythropoiesis; fetal hemoglobin*

Our previous studies demonstrated the existence of regulatory-and-metabolic relationships between the placenta and various fetal organs and tissues, which provides normal development and differentiation of homeostatic systems during embryogenesis [4]. Complicated pregnancy is accompanied by changes in biochemical and/or structural parameters of the fetoplacental system. These disturbances underlie abnormal postnatal development of various organs and systems. Complicated pregnancy is followed by the impairment of erythropoiesis in fetuses and newborn infants. The role of placental factors in the pathogenesis of these changes remains unclear. Previous experiments showed that the placenta synthesizes erythropoietin and its inhibitors [7,11].

Here we evaluated the relationship between erythropoiesis in newborn infants and the state of placentas during normal and complicated pregnancies.

MATERIALS AND METHODS

We examined 33 newborn infants and their placentas. The control group included 12 newborns weighing 3433.30±71.06 g (gestational age more than 39 weeks), whose mothers had no obstetric complications and somatic diseases during pregnancy. The main group included 21 newborns weighing 2540.2±96.2 g (gestational age 39 weeks) and characterized by intrauterine growth retardation (IGR). Gestosis, anemia, and chronic pyelonephritis complicated 72.8, 37.2, and 18.5% pregnancies, respectively.

The state of newborns was estimated by clinical and laboratory assays. Examination of the umbilical and peripheral blood from newborn infants was performed on a Cobos Micros hematological analyzer 1 day after delivery. The concentration of fetal hemoglobin (HbF) in the umbilical blood was measured by alkaline denaturation on a Specol-11 device [1]. Umbilical blood reticulocytes were counted after staining with azure II [1].

Samples for histological assay were taken from the central, intermediate, and marginal placental zones, fixed in 10% neutral formalin, and embedded into paraffin using a Fisher Tissumator device. Paraffin sections (4-5 μ) were stained with hematoxylin and eosin. The placentas were morphologically studied. Morphometric assay of 200 terminal villi from 7 placentas in each group was performed using an G. G. Avtandilov rectangular grid. The placentas of newborn infants with IGR, in which the content of umbilical blood reticulocytes was less than 1%, were subjected to morphometric assay.

Erythropoietic activity of the placental extract was estimated by the biological method [2]. Villous chorion samples were washed with physiological saline to remove the blood and homogenized in a manual homo-

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0007-4888/01/1325-1055$25.00

genizer at the 1:9 tissue/physiological saline weight ratio for 5 min. The solution was centrifuged at 3000 rpm for 10 min. The supernatant (0.1 ml) was mixed with ampicillin (20 mg/ml homogenate) and injected intraperitoneally to 4-week-old JCR mice (10 animals per group). The placentas of newborn infants with IGR, in which the content of umbilical blood reticulocytes was less than 1%, were used to prepare homogenates. Control mice (n=10) received 0.1 ml physiological saline and ampicillin. The animals were killed by cervical dislocation 7 days after injections. Erythropoietic activity was estimated by changes in blood reticulocyte count.

The results were analyzed using Exel software.

RESULTS

Microscopic assay of placentas from newborn infants with IGR revealed pronounced destruction of terminal villi (Table 1). The syncytial epithelium (SE) was characterized by nuclear polymorphism, high count of pyknotic and destructed nuclei, and thinning of the cytoplasm. The total area of SE was lower than in the control. Placental macrophages were found in the stroma of mature intermediate and terminal villi in infants with IGR, but not in the placentas of healthy newborns. The number of capillaries and area of the capillary bed in terminal villi increased compared to the control. Sludge of fetal erythrocytes in the capillary lumen was often seen.

Umbilical blood tests revealed pathological changes in the erythropoietic stem in newborn infants with IGR, including the decrease in reticulocyte count (p<0.05) and increase in HbF concentration (p<0.01, Table 2). Erythrocyte count and total hemoglobin concentration in the umbilical blood tended to decrease in newborns with IGR.

Reticulocyte count in mice receiving the extract from normal placentas was 4.17±0.43%, which surpassed the control by 75.9% (2.37±0.39%, p<0.001). The count of reticulocytes in mice injected with placental extracts from newborns with IGR was 2.51± 0.33%. Therefore, these extracts did not stimulate erythropoiesis.

Published data indicate that complicated pregnancy is accompanied by abnormal development of fetal erythron [3,5,9]. Our results confirm the data that fetal erythropoiesis is impaired during pregnancies complicated by gestosis and IGR [10]. The decrease in umbilical blood reticulocyte count in some newborn infants is probably related to dysfunction of the placenta that regulates fetal erythropoiesis. The placental extract from newborns with IGR did not stimulate erythropoiesis. This can be associated with the impairment of erythropoietin synthesis in the placenta due to destructive changes in its parenchyma and presence of placental tissue inhibitors that block the stimulatory effect of erythropoietin on the bone marrow. Previous experiments showed that the content of fetal blood erythropoietin increases during chronic hypoxia and IGR [8,10]. Therefore, the decrease in reticulocyte count in these children is not related to a deficiency of erythropoietic stimulators.

The placenta is more likely to contain substances inhibiting erythropoiesis in these fetuses. Recent studies showed that hypoxia and destructive changes in the placenta during preeclampsia intensify the synthesis of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) in chorionic villi [7,11]. These factors abolish the stimulatory effect of erythropoietin and suppress the erythropoietic stem [6,12]. Genes of these proinflammatory cytokines contain the DNA sequence homologous to that in an oxygen-dependent enhancer element of the erythropoietin gene. Therefore, synthesis of factors producing opposite effects on the erythron is regulated by the same mechanisms [7]. Placental homogenates from newborn infants with IGR do not stimulate erythropoiesis in laboratory animals, which is probably associated with high contents of TNF-α and IL-1 blocking the effect of placental erythropoietin.

Our results suggest that IGR of fetuses during complicated pregnancies is related not only to the impairment of transport and trophic functions of the placenta, but also to regulatory-and-metabolic disturbances in the mother—placenta—fetus system underlying abnormal development and differentiation of fetal erythron.

TABLE 1. Morphometric Parameters of Placental Villi during Uncomplicated Pregnancy and IGR (M±m, n=7)

Parameter	Uncomplicated pregnancy	IGR
Count of destructed terminal villi, %	6.47±0.99	42.80±5.41*
Epithelial area, %	22.90±1.44	18.10±1.08***
Number of vessels per villus	3.29±0.16	5.59±0.25**
Capillary bed area, %	16.72±1.32	27.80±2.25**

Note. Here and in Table 2: *p<0.001, **p<0.01, and ***p<0.05 compared to uncomplicated pregnancy.

TABLE 2. Umbilical Blood Parameters during Uncomplicated Pregnancy and IGR (*M*±*m*)

Parameter	Uncomplicated pregnancy (*n*=12)	IGR (*n*=21)
Total hemoglobin, g/liter	162.8±1.9	158.2±2.3
Fetal hemoglobin, %	70.4±2.2	88.1±4.3**
Erythrocytes, 10^{12}/liter	5.11±0.44	4.76±0.37
Reticulocytes, %	2.690±0.146	1.370±0.194***

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