Granulocyte/Macrophage Colony-Forming Units from Cord Blood of Premature and Full-Term Neonates: Its Role in Ontogeny of Human Hemopoiesis

DER-CHERNG LIANG, SHEAU-WEN MA, MARIE LIN-CHU, AND CHUNG-CHI LAN

Departments of Pediatrics [D.C.L.], Medical Research [S.W.M. and M.L.C.], and Obstetrics/Gynecology [C.C.L.], Mackay Memorial Hospital, 92, Sec. 2, Chung-San North Road, Taipei, Taiwan, 10449, Republic of China

ABSTRACT. It is still uncertain whether the yolk sac is the only origin of hemopoietic stem cells during fetal development. We studied the level of circulating granulocyte/macrophage colony-forming unit (CFU-GM) from the 23rd week of gestation to fullterm, trying to delineate its role in the ontogeny of human hemopoiesis. Cord blood samples were collected from 45 healthy premature neonates and 91 healthy full-term neonates. CFU-GM assays were performed using a single agar layer system with human placental conditioned medium as a source of colony stimulating activity. The mean numbers of colonies produced from the cord blood samples of the premature and full-term neonates were 116 ± 185 (SD) and $96 \pm 113/2 \times 10^5$ mononuclear cells, respectively. There was no statistical difference between the numbers of colonies of the two groups (Mann-Whitney test, $p > 0.05$). These results indicate that the level of circulating CFU-GM from the 23rd week of gestation to full term is constantly high and add credence to the assumption that CFU-GM are produced not only from the yolk sac but also from other hemopoietic sites. (*Pediatr Res* 24:701-702, 1988)

Abbreviations

CFU-GM, granulocyte/macrophage colony-forming units
HSC, hemopoietic stem cells
BM, bone marrow
CB, cord blood
PB, peripheral blood
MNC, mononuclear cells
YS, yolk sac

The hemopoietic stem cells which form colonies *in vitro* in the presence of colony stimulating activity are designated as colony-forming units in culture and regarded as the committed stem cells of the granulocyte and monocyte-macrophage series (1). During human intrauterine development, hemopoiesis has three phases. In the embryo, erythroblasts arise in the YS. From the 6th wk of gestation, erythropoiesis is transferred to the liver which is the most active site of hemopoiesis from the 6th to the 12th wk of gestation. Although the HSC populations are exclusively erythropoietic, CFU-GM are present in fetal liver in a level

similar to (2) or even higher than (3) that in the adult BM. Hepatic hemopoiesis declines in the 3rd trimester and ceases soon after birth. The onset of BM hemopoiesis occurs during the 15-16th wk of gestation (4). Whether the YS is the only origin of HSC remains to be clarified.

A high level of CFU-GM has been found in human fetal blood from the 12th to the 18th (5) and the 13th to the 21st (4) wk of gestation. CFU-GM has also been noted to be increased in CB of full-term neonates (6-14). In this report, we studied the level of circulating CFU-GM from the 23rd wk of gestation to full term, trying to delineate its role in the ontogeny of human hemopoiesis.

MATERIALS AND METHODS

Materials. CB was collected in sterile tubes containing heparin immediately after delivery and was kept at 4° C for less than 12 h before culture. Altogether, 136 CB samples were collected with 45 being from healthy premature neonates and 91 being from healthy full-term neonates. The gestation ages of the premature neonates ranged from 23 to 36 wk (median, 34 wk), and their birth weights ranged from 650 to 2900 g (median, 2025 g). The BM samples were collected from 52 pediatric patients with normal white blood cell counts, differential counts and normal marrow findings. The BM aspirations were performed for differential diagnosis of thrombocytopenia or unexplained hepatosplenomegaly, or as a staging procedure for solid tumors. The PB samples were collected from 33 healthy adult volunteers.

Methods. CFU-GM assays were performed using a single agar layer system with human placental conditioned medium as a source of colony-stimulating activity (15). CB and BM MNC were cultured at a concentration of 2×10^5 cells and PB at 1×10^6 cells in 1 ml of α -medium containing 10% human placental conditioned medium, 20% fetal calf serum, and 0.3% agar. Duplicate cultures were prepared for each sample and incubated at 37° C in a fully humidified incubator with a constant flow of 7.5% CO₂ in air. The duration of incubation for CB MNC was 10 days and for BM and PB MNC was 7 days. Aggregates of 8-40 cells were counted as clusters and of >40 cells as colonies (16).

Statistical analysis. The Mann-Whitney test was used to determine the significance of the difference between the results of two groups.

RESULTS

The data for granulocyte-macrophage colony and cluster formations of CB, BM, and PB are presented in Table 1. Statistically,

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Correspondence Dr. Der-Cherng Liang, Department of Pediatrics, Mackay Memorial Hospital, 92, Sec. 2, Chung-San North Road, Taipei, Taiwan 10449, Republic of China.

Table 1. Numbers of granulocyte-macrophage colonies and clusters from CB, BM (per 2×10^5 MNC) and PB (per 1×10^6 MNC)*

	CB of premature neonates	CB of full-term neonates	BM	PB
No. of colonies	58, (7-878), (116 $\pm$ 185)	57, (7-731), (96 $\pm$ 113)	173, (20-651), (206 $\pm$ 155)	5, (0-17), (7 $\pm$ 5)
No. of clusters	70, (13-487), (121 $\pm$ 120)	52, (7-725), (110 $\pm$ 148)	281, (47-718), (308 $\pm$ 162)	3, (0-10), (4 $\pm$ 3)
No. of samples	45	91	52	33

* The data are presented as median, (range), (mean $\pm$ SD).

there was no significant difference in the numbers of colonies from CB between premature neonates and full-term neonates ($p > 0.05$). Also, there was no statistical difference in the numbers of clusters from CB between premature neonates and full-term neonates ($p > 0.05$). The pediatric BM gave rise to more colonies than did the CB from both premature neonates ($p < 0.0001$) and full-term neonates ($p < 0.0001$). Also, BM gave rise to more clusters than did the CB from premature neonates ($p < 0.0001$) and CB from full-term neonates ($p < 0.0001$). CB and BM gave rise to many more colonies and clusters than did PB. The colony and cluster numbers/ml of CB were counted on 20 samples based on the concentrations of MNC in CB. And there were 3989 ± 5606 colonies/ml and 4127 ± 4510 clusters/ml (mean $\pm$ SD) formed.

DISCUSSION

In chicken embryos, the HSC of intraembryonic origin are scattered all over the body and are especially numerous in the dorsal mesentery in the vicinity of the aorta (17). In quail embryos, the foci of the intraembryonic HSC are localized in the angle of the duct of Cuvier and anterior cardinal vein (17). In mice, an earlier study showed that YS was the only source of CFU-GM formation, and these HSC migrated via PB to colonize other hemopoietic organs (18). However, Barker (19) demonstrated a maximum number of CFU-GM in PB of 12-day embryonic mice, a stage at which the liver shows a fully expressed hemopoietic capacity. This finding may indicate that the liver is capable of adding HSC to PB (20). The observation of a constantly high number of CFU-GM in PB of mice during embryonic development till the end of gestation indicates that other hemopoietic organs are also producers of HSC (20).

Our results show that in humans the level of circulating CFU-GM from the 23rd wk of gestation to full term is constantly high. Since a high level of CFU-GM was also found in human fetal blood during the 12-18th (5) and the 13-21st (4) wk of gestation, it is concluded that in humans, just as in mice, there is a high level of circulating CFU-GM from the 12th wk of gestation to full term. Therefore, because CFU-GM in mice are produced in YS and other hemopoietic sites (20), it is assumed that this is the same in humans.

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