Analysis of Granulomonocytic Precursor Cells from Fetal Liver Used for Maintenance Therapy in Children with Gaucher's Disease

I. V. Dubrovina, E. A. Kopyl'tsova, L. A. Cheredeeva, V. F. Kudryashova, Yu. A. Blidchenko, N. A. Torubarova, and G. T. Sukhikh

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 7, pp. 78-80, July, 1999
Original article submitted June 24, 1996

Granulocyte-macrophage precursor cells of fetal liver are characterized by high proliferative capacity. Being administered to children with Gaucher's disease, these cells provide a short-term maintenance of the monocyte/macrophage pool with normal level of glucocerebrosidase.

Key Words: *Gaucher's disease; granulocyte-macrophage precursor cells*

Gaucher's disease is a genetic disorder caused by insufficiency of lysosomal enzyme glucocerebrosidase. Intravenous administration of a freshly prepared suspension of fetal liver cells containing monocytes and macrophages with maximum activity of lysosomal glucocerebrosidase is used for the therapy of this disease [2]. Granulocyte-macrophage precursor cells (GMPC) from fetal liver are suggested to proliferate in the body and maintain the population of healthy monocytes [4].

Here we studied GMPC of fetal liver tissues and analyzed the effects of various growth factors on hemopoietic activity of fetal liver cells.

MATERIALS AND METHODS

Livers of 11 fetuses (16-22 weeks of gestation) weighing 200-500 g that were obtained from 16-40-year-old healthy women. All tests for viral infections (hepatitis B, cytomegalovirus, herpes virus, and human immunodeficiency virus) and toxoplasmosis and Wassermann test were negative. The period from liver sampling to the analysis did not exceed 4 h.

The colony-forming potency of fetal liver GMPC was studied in Petri dishes by Pike and Robinson's double layer agar technique with modifications [1]. A total of 138 cultures were analyzed.

The liver was aseptically homogenized in RPMI-1640 medium; it usually consisted of $3.5\text{-}10.5\times10^9$ cells. Cell viability assayed by 0.2% trypan blue exclusion was 75%.

Human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), human recombinant interleukin-3 (IL-3), their combination, peripheral blood leukocytes pooled from hematologically healthy donors (feeder), umbilical cord-conditioned medium obtained by the method of A. Muller (1989), and non-inactivated serum of patients with chronic glomerulonephritis were used as GMPC growth stimulators.

The number of colonies and clusters per 2×10^5 cells was calculated on day 7 in culture, and cloning efficiency (total number of colonies and clusters) was determined.

Morphological analysis of colonies and clusters was performed on a microscope slide. Myeloperoxidase was assayed by the method of Gram—Knoll followed by Romanovsky—Giemsa staining.

RESULTS

Standard liver culture (on the feeder) of a 16-22-week fetus contained 31.8±4.0 (20-49) GMPC. This level was 2 times lower ($p<0.01$) than in the bone marrow

Institute of Pediatrics, Russian Academy of Medical Sciences; International Institute of Biomedicine, Moscow

0007-4888/99/0007-0727$22.00

of a child with normal hemopoiesis (Fig. 1). In all liver cultures, colony growth 2-fold surpassed cluster growth. The ratio of colony-forming to cluster-forming capacity of the bone marrow cultures was 0.8± 0.1. Similar results were obtained in experiments with various growth factors.

Granulocyte-macrophage colonies from the liver contained more cells (100-500) than bone marrow colonies (below 100).

These indexes (the prevalence of colonies in cultures and their greater size) indicate higher proliferative capacity of liver precursor cells in comparison with bone marrow precursor cells.

The ratio of compact (granulocytic) to diffuse (macrophageal) colonies in the standard culture (on the feeder) was 1.3. Morphocytochemical study showed that practically all colonies (independently on their shape) were mixed and contained monocytes and granulocytes at various maturation stages. Macrophage colonies were also found. The majority of clusters and small colonies were presented by eosinophils and macrophages.

Various growth stimulators induced similar effects on liver GMPC. The most pronounced response was induced by GM-CSF (cloning efficiency 20-59 per 2×10^5 cells) and the combination of GM-CSF and IL-3 (22-54/2×10^5 cells).

Stimulation of cloning by IL-3 (a universal growth factor for all types of hemopoietic precursors) was unexpectedly low (Fig. 2), while the combination of IL-3 and GM-CSF produced considerable effects. The data suggest that fetal liver GMPC are more sensitive to GM-CSF than to IL-3.This is consistent with the idea that receptors of granulocyte precursors and mature granulocytes display high affinity to GM-CSF but not to IL-3. Receptors on monocytes can selectively bind GM-CSF and IL-3. However, only high concentrations of IL-3 stimulate their proliferation [3].

An individual analysis showed differences in the sensitivity to various growth factors in fetal liver GMPC obtained from various fetuses. The colony-forming potency of the liver from fetus 68 was characterized by high spontaneous growth activity (cloning efficiency 18.8 per 2×10^5 cells). Liver cells from fetus 65 were the most sensitive to the presence of growth factors in umbilical cord-conditioned medium. Liver cells from fetus 69 displayed the highest sensitivity to IL-3: the colony-forming potency and the efficiency of cloning were 128, cluster growth was absent, and the number of cells in colonies was more than 200. In the

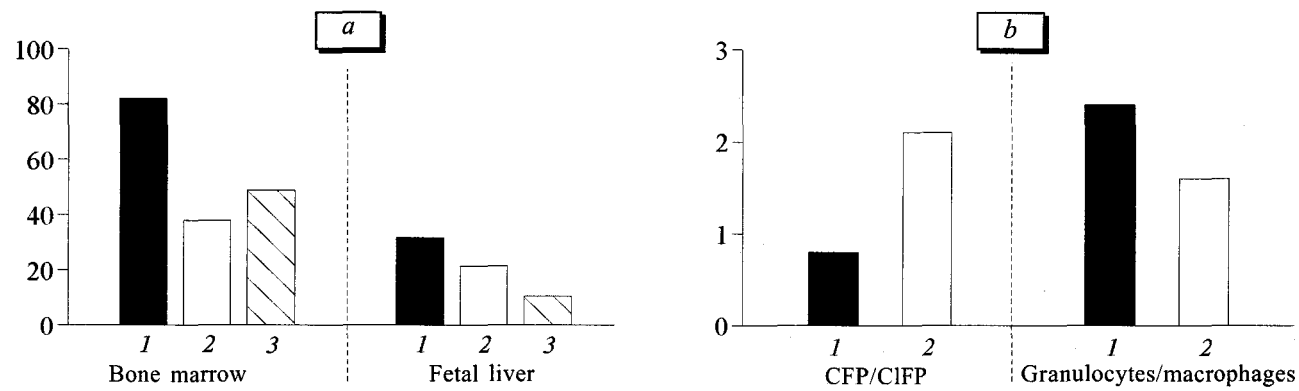

Fig. 1. Colony-forming capacity of bone marrow and fetal liver cells. *a*) cloning efficiency (*1*) and numbers of colonies (CFC, *2*) and clusters (ClFC, *3*) per 2×10^5 cells; *b*) bone marrow (*1*) and fetal liver (*2*).

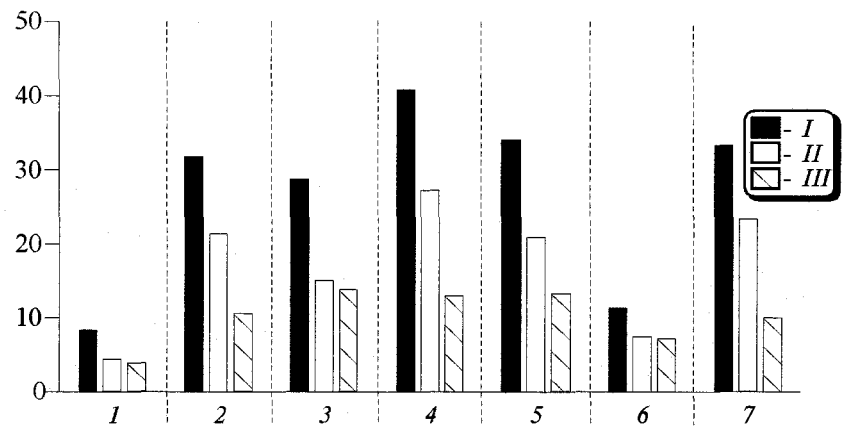

Fig. 2. Colony-forming capacity of fetal liver cells: spontaneous (*1*) and stimulated with feeder (*2*), umbilical cord-conditioned medium (*3*), granulocyte-macrophage colony-stimulating factor (GM-CSF, *4*), GM-CSF and interleukin-3 (IL-3, *5*), IL-3 (*6*), and blood serum (*7*). Cloning efficiency (*I*) and numbers of colonies (*II*) and clusters (*III*) per 2×10^5 cells.

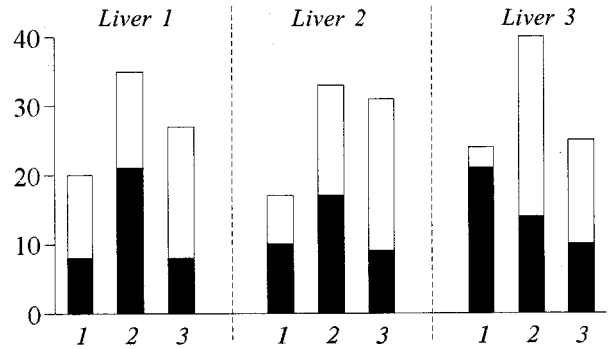

Fig. 3. Effects of growth factors on the number and ratio of granulocytic (dark bars) and macrophageal (light bars) colonies in the liver. Stimulation with feeder (*1*), granulocyte-macrophage colony-stimulating factor (GM-CSF, *2*), and GM-CSF+interleukin-3 (*3*).

presence of both GM-CSF and IL-3, the maximum cloning efficiency was found in liver cells from fetus 75.

The analysis of the size and composition of colonies showed that the largest colonies containing 100-500 cells appeared under the effects of GM-CSF, while IL-3 induced the formation of colonies containing less than 100 cells. Blood serum from patients with glomerulonephritis induced the formation of normoblast clusters in cultures.

Effects of growth factors on the ratio of granulocytic to macrophageal colonies in cultures were studied. Morphological and cytochemical analyses showed individual variations in the composition of colonies in the presence of different growth factors (Fig. 3). Cultures consisting of mainly granulocytic or macrophageal colonies or containing nearly similar number of these colonies were obtained under standard conditions (on the feeder). GM-CSF increased the number of colonies of both types and, therefore, the efficiency of cloning. In some cases, macrophageal colonies prevailed. However, the combination of GM-CSF and IL-3 increased the number of macrophageal colonies.

REFERENCES

1. B. V. Afanas'ev and V. A. Almazov, *Human Hemopoietic Precursor Cells* [in Russian], Leningrad (1985).
2. G. A. Grabowski, *Mol. Med. Ther.*, **2**, No. 3, 2-7 (1994).
3. A. F. Lopez, M. J. Elliott, J. Woodcock, and M. A. Vadas, *Immunol. Today*, **13**, No. 12, 495-500 (1992).
4. J-L. Touraine, M-G. Roncarollo, R. Bacchetta, *et al.*, *Bone Marrow Transplant.*, **11**, No. 1, 119-121 (1993).