Two Types of Immature Megakaryocytic Series in the Human Fetal Liver

Iwao EMURA, Masao SEKIYA and Yoshihisa OHNISHI

Department of Pathology (Prof. Y. OHNISHI), Niigata University School of Medicine, Japan

Received June 23, 1982

Summary. Megakaryocytes in the liver obtained from 185 human embryos and fetuses during the period from 28 days to 22 weeks of ovulation were investigated by light and electron microscopy.

The early hepatic megakaryoblasts and the early hepatic promegakaryocytes at stage I observed in the intercellular spaces of the hepatocytes until 10 weeks of ovulation (early stage of hepatic hemopoiesis) were larger than the late hepatic megakaryoblasts and the late hepatic promegakaryocytes at stage I observed after 10 weeks of ovulation (late stage of hepatic hemopoiesis). The chromatin of the former two cells was finely dispersed, whereas that of the latter two showed moderate central and peripheral clumping.

These findings seem to indicate that the progenitor cells of the megakaryoblasts and the hemopoietic stem cells in the liver in the early stage of hepatic hemopoiesis morphologically differ from those in the liver in the late stage, and that the megakaryocytes in the liver until 10 weeks of ovulation differ in maturation course from those after this ovulation stage.

Experimental investigations of colony forming units have verified the existence of pluripotential hemopoietic stem cells (CFU-S) in experimental animals (TILL and McCulloch, 1961; BECKER, McCulloch and TILL, 1963). Recent studies on mixed colony formation have revealed the presence of human pluripotent hemopoietic progenitors (CFU-GEMM) (FAUSER and MESSNER, 1978, 1979). The ultrastructure of CFU-S is considered to resemble that of small lymphocytes (De BEKKUM et al., 1971; DICKE, NOORD and BEKKUM, 1973). Furthermore, it has been shown that the morphological features of the megakaryoblasts in the bone marrow of guinea pigs are similar to those of lymphocytes (FEDORKO, 1978). From these findings, the ultrastructures of the progenitor cells of megakaryoblasts and the hemopoietic stem cells in the human embryonic or fetal liver are also presumed to resemble those of lymphocytes. In our study on hemopoiesis in human embryonic liver, however, we could not find any lymphoid cells until 40 days after ovulation. This research was conducted to reveal the precise ultrastructure of immature megakaryocytes during hemopoiesis in the human liver.

MATERIALS AND METHODS

The hepatic tissues examined were obtained from 109 human embryos from 28 to 49 days after ovulation and 76 human fetuses ranging from 8 to 22 weeks of ovulation by legal abortion from healthy women.
Light microscopy: Eighteen embryos between 4 to 24 mm crown rump (C. R.) length (estimated age: 30 to 49 days after ovulation) and hepatic tissue of 34 fetuses (50 to 154 days of ovulation) were fixed in 10% neutral formalin and then embedded in paraffin. Every fifth serial section was stained with hematoxylin-eosin, and the rest were subjected to silver impregnation and periodic acid-Schiff reaction.

Transmission electron microscopy: Ninety-one embryos, including a 21 somite embryo of 3 mm C. R. length (estimated age, 27 ± 1 days), and the hepatic tissues of 76 fetuses were fixed as soon as possible after legal abortion in the fixative, which consisted of 2% glutaraldehyde in 0.1 M phosphate buffer solution, pH 7.5, at 4°C for 2 hrs. In the fixative the embryos and livers were divided into small pieces using a dissection microscope. The tissue pieces were then rinsed in 0.1 M phosphate buffer solution, pH 7.5, and postfixed in 1% osmium tetroxide at 4°C for 2 hrs. All the specimens were dehydrated and embedded in Epon. Ten sets 30-40 serial ultrathin sections were routinely prepared at an interval of 15-20μm from a block of each case. Ultrathin sections were counterstained with uranyl acetate and lead citrate. The sections were examined with a Hitachi HS-9 electron microscope.

RESULTS

I. Light microscopic observation of hepatic hemopoiesis

For embryo within the first month, no hemopoietic cells were found in the hepatic parenchyma, and all blood cells in the sinusoids were primitive erythroblasts of yolk

Fig. 1. An isolated hemopoietic cell in the intercellular spaces in the liver of a human embryo, about 33 days after ovulation (arrow). The nucleus is indented and has a distinct nucleolus. The chromatin is finely dispersed. × 850
Two Human Fetal Megakaryocytic Series

A small number of isolated hemopoietic cells were first identified in the intercellular spaces among the hepatocytes of an embryo of 6.2 mm C. R. length (estimated ovulation age: 33 days) (Fig. 1). With the embryo's development, the number of erythrocytes and megakaryocytes gradually increased in the hepatic parenchyma. Forty days after ovulation, hemopoiesis in the liver seemed completed (Fig. 2).

II. Electron microscopic observation of hepatic hemopoiesis

Until 40 days after ovulation, the greater part of hemopoietic cells in the hepatic parenchyma were erythrocytic; megakaryocytes were frequently found, but granulocytes were hardly observed. All the immature hemopoietic cells in the liver during the period 33 to 40 days after ovulation ranged in size from about 13 to 15 μm; the chromatin of these cells was finely dispersed.

A very small number of lymphoid cells with a nucleus showing central and peripheral clumps of chromatin in various degrees was first disclosed in the intercellular spaces of hepatocytes of an embryo of 13 mm C. R. length (estimated ovulation age: 40 days). These lymphoid cells quite slowly increased in number with the development of embryos.

Megakaryoblasts and promegakaryocytes at stage I that resembled lymphoid cells were first found in the intercellular spaces of embryonic hepatocytes at about the tenth week of ovulation. However, these cells were small in number throughout all stages of hepatic hemopoiesis.

Frequently, megakaryocytes were found beneath the endothelia of sinusoids, and immature megakaryocytes were often found in the sinusoidal lumina. Desmosome-like

Fig. 2. A human embryonic liver, about 50 days after ovulation. Erythroblasts form a greater part of the hemopoietic cell population, and a few megakaryocytes are found among them. ×850
structures, such as those found between the erythroblasts and the hepatocytes (Fukuda and Sato, 1971), were not detected between the megakaryocytes and the hepatocytes.

III. Cells of the megakaryocytic series

In this paper, the following nomenclature for megakaryocytic cells is based upon that of previous works (Fedorko, 1978; Bessis, 1973; Baldini and Ebbe, 1974): The adjective "early hepatic" is applied to the immature megakaryocytes observed in the liver at the early stage to hepatic hemopoiesis, whereas, the adjective "late hepatic" to the immature megakaryocytes at the late stage, since the immature cells of megakaryocytic lineage in the liver at the early stage differ in ultrastructure from the immature cells at the late stage. As for the megakaryocytes that are more mature than promegakaryocytes at stage II, we could not notice any distinct morphological differences between the megakaryocytes in the liver before 10 weeks of ovulation and those after 10 weeks. Therefore the adjectives "early hepatic" or "late hepatic" are not applied to either promegakaryocytes at stage III or mature megakaryocytes.

A. Immature cells of an early hepatic megakaryocytic series

1. Early hepatic megakaryoblasts (Fig. 3): Ranging from 13 to 22 μm in diameter (mean: 18 μm), these cells showed high nucleo-cytoplasmic ratio. The oval or lobulated nucleus possessed one or two large nucleoli. Nuclear pores were numerous and the chromatin was finely dispersed. The cell margin was distinct. The cytoplasm showed an initial formation of the demarcation membrane system (DMS) and the narrow marginal zone. The cytoplasmic rim contained numerous polyribosomes, a small number

Fig. 3. An early hepatic megakaryoblast in the hepatic parenchyma of a human embryo, about 5 weeks after ovulation. The chromatin is finely dispersed in the nucleus. Note the earliest stage of DMS formation (arrow). × 7,500
of slender cisternae of rough endoplasmic reticulum (r-ER), a moderate number of mitochondria and a pair of centrioles. The Golgi apparatus was composed of a small number of vesicles and lamellar cisternae. No characteristic granules were found.

2. Early hepatic promegakaryocytes at stage I (Fig. 4): These cells ranged in size from 14 to 26 \( \mu m \) (mean: 19 \( \mu m \)). The nucleocytoplasmic ratio decreased as the nuclear lobulation became more obvious. The nucleoli were prominent in each nuclear lobe. The chromatin was finely dispersed and the nuclear pores were numerous. The Golgi apparatus was composed of small vesicles and lamellar cisternae. Polyribosomes were numerous, and mitochondria tended to gather around the Golgi apparatus. The cisternae of r-ER were slightly distended and contained an amorphous material. A small number of characteristic granules up to 0.2 \( \mu m \) in diameter were found to be elaborated in the lateral dilatation of the Golgi apparatus and spread over the cytoplasm. A few centrioles were found in the Golgi area. Small mazy clumps of DMS were observed close to the cell surface. The narrow marginal zone became wider.

3. Early hepatic promegakaryocytes at stage II (Fig. 5): These cells measured 16–30 \( \mu m \). The nucleus was multilobulated with a slight margination of chromatin. Nucleoli were observed in each nuclear lobe; nuclear pores were numerous. The marginal zone became wider and small bullous pseudopodia were present in this zone. Polyribosomes, mitochondria, cisternae of r-ER and small characteristic granules increased in number. The large Golgi apparatus was composed of numerous small vesicles and lamellar cisternae. Two or more centrioles were often found in the large Golgi area. Mazy clumps of the DMS became larger and were located near the Golgi apparatus.
I. EMURA, M. SEKIYA and Y. OHNISHI:

B. Immature cells of a late hepatic megakaryocytic series

1. *Late hepatic megakaryoblasts* (Fig. 6): Ranging from 8 to 10 μm (mean: 9 μm) in diameter, these cells showed a high nucleo-cytoplasmic ratio. Generally, the nucleus was irregular in contour with occasional indentations and had one or two prominent nucleoli. The chromatin showed moderate central and peripheral clumping. Nuclear pores were small in number. The cytoplasm was abundant in the free polyribosomes and single ribosomes. The Golgi apparatus was mainly composed of a few small vesicles. Ten to fifteen mitochondria per section frequently gathered around the small Golgi apparatus, and a pair of centrioles was present in the Golgi area. Elements of the r-ER were rare. The formation of the DMS was in its earliest stage.

2. *Late hepatic promegakaryocytes at stage I* (Fig. 7): The size of these cells ranged from 9 to 12 μm (mean: 10 μm). Nuclear lobulation became obvious. The chromatin showed moderate central and peripheral clumping, and nucleoli existed in each nuclear lobe. Nuclear pores increased in number. The cytoplasm had numerous polyribosomes and slender cisternae of r-ER. The Golgi apparatus was composed of many small vesicles and lamellar cisternae. A small number of characteristic granules were seen to be produced by the lateral dilatation of the Golgi apparatus. One or more centrioles were present in the Golgi area; twelve to seventeen mitochondria per section gathered around it.

3. *Late hepatic promegakaryocytes at stage II* (Fig. 8): These cells measured 14–30 μm in diameter. The nucleus was multilobulated with numerous nuclear pores. The

Fig. 5. An early hepatic promegakaryocyte at stage II in the hepatic parenchyma of a human embryo, about 6 weeks after ovulation. Many specific granules and a mazy clump of DMS are present around the Golgi apparatus. × 3,900
chromatin showed slight or moderate margination. Nucleoli were found in each nuclear lobe. The marginal zone became wider and had small bullous pseudopodia. The large Golgi apparatus was composed of numerous small vesicles and lamellar cisternae. Three or more centrioles were often found in the Golgi area. The cytoplasm displayed numerous polyribosomes, slender cisternae of r-ER, and mitochondria. Small characteristic granules increased in number. The mazy clumps of DMS became larger and were located near the Golgi apparatus.

C. Promegakaryocytes at stage III and mature megakaryocytes

1. Promegakaryocytes at stage III (Fig. 9): These cells ranged in size from 20 to 30 μm. The marginal zone became wider and large hyaloplasmic pseudopodia were present. They were sharply delineated by the plasma membrane and generally had a rather hemogenous structure. They were devoid of mitochondria, polyribosomes, cisternae of r-ER, DMS and of characteristic granules. Almost all organelles, including clumps of DMS and a lobulated nucleus, gathered in the central area of the cell. The polyribosomes, mitochondria, cisternae of r-ER and characteristic granules increased in number. The Golgi apparatus was composed of numerous small vesicles and lamellar cisternae. The nucleus showed a slight margination of chromatin and the nucleoli were found in each nuclear lobe.

2. Mature megakaryocytes (Fig. 10): These cells ranged from 20 to 40 μm in size. The nucleus was multilobulated with a slight to moderate margination of the chromatin.
The DMS spread over the cytoplasm and the three zones—marginal, intermediate and perinuclear—of the cytoplasm became obvious. The marginal zone narrowed with cell maturation and the hyaloplasmic pseudopodia decreased in number and size. The wide intermediate zone contained a greater part of the organelles. The characteristic small granules increased in number, whereas the polyribosomes and the cisternae of r-ER decreased with cell maturation. The Golgi apparatus was present, close to the nucleus. The perinuclear zone gradually became narrower with cell maturation.

DISCUSSION

The maturation of megakaryocytes has been studied ultrastructurally by several investigators. The greater part of the studies have been performed on megakaryocytes in the bone marrow of experimental animals, and only a few articles concerning the maturation of human megakaryocytes are available. The investigation of the human fetal liver revealed the precise ultrastructure of immature megakaryocytes.

Bessis (1956) described megakaryoblasts as about 30 μm in diameter with a single oval nucleus, and observed that these cells formed 5–15% of megakaryocyte population of human bone marrow. However, it is only in the human fetal liver (Zamboni, 1965), in experimental animals (MacPherson, 1971; Fedorko, 1978) and in human megakaryoblastic acute leukemia (Breton-Gorius, 1978) that cells corresponding to the megakaryoblast have been identified by electron microscopy. Both megakaryoblasts described by
ZAMBONI (1965) and the most immature cells described by MACPHERSON (1971) show early signs of characteristic granule formation in the Golgi apparatus. The size of these cells ranges from 13 to 15 μm and their nuclei are lobulated with slight margination of the chromatin. We speculate that these cells are equivalent to the late hepatic promegakaryocytes at stage I or stage II of our classification.

Using a histochemical approach, JACKSON (1974) showed that the precursor cells of megakaryocytes in rat bone marrow were smaller than those that were morphologically recognizable as megakaryocytes. FEDORKO (1978), employing immunohistochemical procedures, revealed that the megakaryoblasts in guinea pig bone marrow measured 8–10 μm in diameter and the nuclear chromatin showed moderate central and peripheral clumping. In addition, BRETON-GORIUS (1978), using the cytochemical method, reported that the megakaryoblasts in human megakaryoblastic acute leukemia possessed a uniform size of 10 μm and that the chromatin of these cells showed moderate central and peripheral clumping. All these megakaryoblasts resemble the late hepatic megakaryoblast but not the early ones.

The cells shown in Figures 3 and 6 have narrow cytoplasmic projections extending above and below the cell surface. These structures are similar to those at the earliest stage of DMS formation reported by MACPHERSON (1972). Therefore, we judged that these structures were created in the earliest stage of DMS formation and that the cells in Figures 3 and 6 were megakaryocytic. Specific granules were not found in these cells, and so we considered the cells to be in the same maturation stage, and categorized them as megakaryoblasts. It was concluded that the cells shown in Figures 4 and 7...
were also in the same maturation stage and that these were more mature than the cells shown in Figures 3 and 6 respectively because a small number of characteristic granules were found by lateral dilatation of the Golgi apparatus.

Though no distinctive morphological differences could be noticed between promegakaryocytes at stage III, mature megakaryocytes in the liver at the early stage of hepatic hemopoiesis and those in the liver at the late stage, there are two major morphological differences between the immature cells of an early hepatic megakaryocytic series and those of a late one. The first is that the ultrastructure of the nuclei of the early hepatic megakaryoblasts and the early hepatic promegakaryocytes at stage I are definitely different from those of the late hepatic megakaryoblasts and the late hepatic promegakaryocytes at stage I, since the nuclear chromatin of the former cells was finely dispersed, whereas that of the latter cells showed moderate central and peripheral clumping. The difference of the chromatin pattern does not seem to be related to the cell cycle difference because the cells shown in Figures 3–10 were not in mitotic stages. The second is that the early hepatic megakaryoblasts and the early hepatic promegakaryocytes at stage I are apparently larger than the late-stage hepatic megakaryoblasts and the late hepatic promegakaryocytes at stage I, respectively.

It is not fully understood whether the hemopoietic stem cells in the human embryonic liver migrate from the yolk sac or arise from within the embryo (Moore and Metcalf, 1970; Dieterlen-Lievre, Beaupain and Martin, 1979). If only one kind of
hemopoietic stem cells exists in the human embryonic liver the megakaryoblasts and promegakaryocytes at stage I in the liver at both the early and the late stages of hepatic hemopoiesis should possess almost the same size and similar ultrastructure. Morphologically, however, the early hepatic megakaryoblasts were clearly distinguishable from the late ones and lymphoid cells were not found in the liver before 40 days of ovulation. These findings seem to indicate that both the precursor cells of megakaryoblasts and the hemopoietic stem cells in the liver at the early stage are morphologically different from those in the liver at the late stage, and that the maturation course of the early hepatic megakaryocytic series is different from that of the late hepatic megakaryocytic series.

Acknowledgment. Appreciation is expressed to the doctors of obstetrics and gynecology at Takeyama Hospital and Saiseikai Niigata Hospital for providing cases. Thanks are also due to Mr. T. HASEGAWA, Mr. K. SATO and Mr. S. MOMOZAKI, Department of Pathology, School of Medicine, Niigata University for their technical assistance.

REFERENCES

Baldini, M. G. and S. Ebbe (ed.): Platelets, production, function, transfusion and storage. Grune & Stratton, New York-San Francisco-London, 1974.
Becker, A. J., E. A. McCulloch and J. E. Till: Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197: 452-454 (1963).

Bekkum, D. W., M. J. van Noord, B. van Maat and K. A. Dicke: Attempts at identification of hemopoietic stem cells in mouse. Blood 38: 547-558 (1971).

Bessis, M. (ed.): Cytology of the blood and blood forming organs. Grune & Stratton, New York, 1956.

Bekkum, D. W., M. J. van Noord, B. van Maat and K. A. Dicke: Attempts at identification of hemopoietic stem cells in mouse. Blood 38: 547-558 (1971).

Breton-Gorius, J., F. Reyes, G. Duhamel, A. Najman and N. C. Gorin: Megakaryoblastic acute leukemia: Identification by the ultrastructural demonstration of platelet peroxidase. Blood 51: 45-60 (1978).

Dicke, K. A., M. J. van Noord and D. W. van Bekkum: Attempts at morphological identification of hemopoietic stem cell in rodents and primates. Exp. Hematol. 1: 36-45 (1973).

Dieterlen-Lievre, F., D. Beaupain and C. Martin: Potentialities and migrations of hemopoietic stem cells of yolk sac and intraembryonic origins, studied in avian chimeras obtained by blastoderm recombination. In: (ed. by) N. LeDouarin: Cell lineage, stem cells and cell determination. North-Holland Publishing Company. Amsterdam-New York-Oxford, 1979. (p. 175-189).

Fauser, A. A. and A. H. Messner: Granuloerythropoietic colonies in human bone marrow, peripheral blood and cord blood. Blood 52: 1243-1248 (1978).

Fedorko, M. E.: Morphologic and functional observation on bone marrow megakaryocytes. In: (ed. by) R. Silber, J. Lobue and A. S. Gordon: The year in hematology 1978. Plenum Medical Book Company, New York and London, 1978 (p. 171-209).

Fukuda, K. and H. Sato: Desmosome, cilia, and peculiar structure of membranes in erythroblasts of human fetal liver. Virchows Arch. Abt. B 7: 309-313 (1971).

Jackson, C. W.: Some characteristics of rat megakaryocyte precursors identified using cholinesterase as a marker. In: (ed. by) M. G. Baldini and S. Ebbe: Platelets production, function, transfusion and strage. Grune & Stratton. New York-San Francisco-London, 1974. (p. 33-40).

Macpherson, G. G.: Development of megakaryocytes in bone marrow of the rat: an analysis by electron microscopy and high resolution autoradiography. Proc. Roy. Soc. Lond. B 177: 264-274 (1971).

Moore, M. A. S. and D. Metcalf: Ontogeny of the haemopoietic system: Yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. Brit. J. Haematol. 18: 279-296 (1970).

Till, J. E. and E. A. McCulloch: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Rad. Res. 14: 313-322 (1961).

Zamboni, L.: Electron microscopic studies of blood embryogenesis in humans. II. The hemopoietic activity in the fetal liver. J. Ultrastr. Res. 12: 525-541 (1965).