Two Types of Progenitors of the Granulocyte Series in the Human Embryonic Liver

Iwao EMURA, Masao SEKIYA and Yoshihisa OHNISHI

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

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Summary. The neutrophilic granulocyte series in the human liver obtained from 109 embryos between 28 to 49 days after ovulation and 76 fetuses between 8 to 22 weeks of gestation was investigated by light and electron microscopy.

Hemopoietic cells, considered progenitors of the granulocyte series, and undifferentiated cells first appeared in the intercellular spaces of mesenchymal cells around the ductus venosus of an embryo of 14 mm crown rump length (estimated age: 40 days after ovulation). The early hepatic myeloid progenitor cells which appeared by 50 days of gestation (early stage of hepatic granulopoiesis) differed in ultrastructure from the late hepatic myeloid progenitor cells which first occurred after 50 days of gestation (late stage of hepatic granulopoiesis).

These findings indicate that, morphologically, two kinds of hemopoietic stem cells exist in the human embryonic and fetal liver, and that the hepatic granulocyte series in the early stage differs in its process of maturation from that in the late stage.

Endogenous peroxidase activity was demonstrated in small granules of the late hepatic myeloid progenitor cells which appeared lymphoid in ultrastructure.

Experimental investigations of colony forming units in hemopoiesis have verified the existence of pluripotential hemopoietic stem cells (CFU-S) in experimental animals (Till and McCulloch, 1961; Becker, McCulloch and Till, 1963). The CFU-S is known to resemble small lymphocytes in ultrastructure (De Bekkum et al., 1971; Dicke, Noord and Bekkum, 1973).

In our previous paper, it was reported that the hemopoietic stem cells of the early hepatic megakaryocytic series morphologically differed from those of the late hepatic megakaryocytic series and that the maturation pathway of the former series was different from that of the latter series (Emura, Sekiya and Ohnishi, 1983). Should the stem or precursor cells of the granulocyte series in the early stage prove to be morphologically different from those in the late stage, our hypothesis will be further supported.

The localization of myeloperoxidase in immature non-neoplastic hemopoietic cells of the granulocyte series remains an area of inquiry.

This research was conducted in order to elucidate the precise ultrastructure of the granulocyte series in the human embryonic liver along with the localization of myeloperoxidase activity in these cells.
MATERIALS AND METHODS

The hepatic tissues examined were obtained from 109 human embryos 28 to 49 days after ovulation and 76 human fetuses at 8 to 22 weeks of gestation by legal abortion from healthy women.

Light microscopy: Eighteen embryos between 4 to 20 mm crown-rump (C. R.) length (estimated age: 30 to 49 days after ovulation) and hepatic tissues of 34 fetuses (50 to 154 days of gestation) were fixed in 10% neutral formalin and embedded in paraffin. Every fifth serial section was stained with hematoxylin-eosin, and the rest subjected to silver impregnation, naphthol AS-D chloroacetate esterase and periodic acid-Schiff reaction.

Transmission electron microscopy: Ninety-one embryos, including a 21 somites embryo of 3 mm C. R. length (estimated ovulation age, 27 ± 1 days), and 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 fixation step, the embryos and livers were divided into small pieces using a dissection microscope. Tissue pieces, including the ductus venosus, were selected and rinsed in 0.1 M phosphate buffer solution, pH 7.5, and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer solution, pH 7.5, at 4°C for 2 hrs. The specimens were dehydrated and embedded in Epon. Ten sets of 30–40 serial ultrathin sections were routinely prepared at intervals of 15–20 μm 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.

Fig. 1. Peroxidase activity in the cells of granulocytic series in the intercellular spaces around the ductus venosus of a human embryo about 50 days after ovulation. × 250
Endogenous peroxidase: Ultrastructural peroxidase reactions were performed for the hepatic granulocyte series. A part of the small pieces of the hepatic tissues—including the ductus venosus—were washed in 0.05 M Tris-HCl buffer pH 7.4. Thin sections, about 30 μm in thickness, were prepared from the pieces using a tissue sectioner. They were incubated in the medium consisting of 5 mg of 3,3' diaminobenzidine tetrahydrochloride (DAB, Nakarai Chemicals LTD) in 10 ml 0.05 M Tris-HCl buffer pH 7.4 for 30 min at room temperature, and rinsed in 0.05 M Tris-HCl buffer pH 7.4. They were then incubated in a medium containing 0.001% H₂O₂ in 0.05 M Tris-HCl buffer pH 7.4 for 10 min at room temperature. After being washed all the samples were postfixed in 1% osmium tetroxide at 4°C for 2 hrs and dehydrated and embedded in Epon. Thin sections cut with a diamond knife were examined before and after staining with lead citrate and uranyle acetate under a Hitachi HS-9 electron microscope. Tissues serving as controls were incubated in a medium without DAB.

RESULTS

I. Light microscopic observation of hepatic granulopoiesis

Until 40 days of ovulation, the major part of hemopoietic cells among hepatocytes in the embryonic liver were erythrocytic or megakaryocytic, and no hemopoietic cells were observed among the mesenchymal cells around the ductus venosus.

In an embryo of 14 mm C. R. length (estimated ovulation age: 40 days), a small

Fig. 2. An early hepatic myeloid progenitor cell around the ductus venosus of a human embryo, about 42 days after ovulation. The cisternae of rough endoplasmic reticulum are short in profile and few in number. The cytoplasm contains a moderate amount of polyribosomes. The chromatin is finely dispersed in the nucleus. ×8,400
number of undifferentiated hemopoietic cells and the granulocyte series were first disclosed in the intercellular spaces of the mesenchymal cells around the ductus venosus.

With the embryo's development, the granulocyte series increased in number not only around the ductus venosus but also around the large arteries in the liver (Fig. 1). The granulocyte series that were scattered among hepatocytes or in the other regions also increased in number. Hemopoiesis was not observed in any marrow until 10 weeks of gestation. Within 8 weeks of gestation, the lymph nodes had not yet formed, nor were any lymphoid cells found in the epithelial rudiment of the thymus.

II. **Electron microscopic observation of hepatic granulopoiesis**

The undifferentiated hemopoietic cells that first appeared in the intercellular spaces of mesenchymal cells around the ductus venosus ranged in size from 13 to 15 μm. The cells that resembled small lymphocytes in ultrastructure were not detected simultaneously. The granulocyte series increased within a week, and the undifferentiated hemopoietic cells so rapidly decreased in number that they could not be detected around the ductus venosus after 7 weeks of gestation.

At the earliest stage of hepatic development, mesenchymal cells around the ductus venosus were in close intimate contact with adjacent hepatocytes. But after 8 weeks of gestation, the hepatic parenchymal cells came to be bordered by a basement membrane from the mesenchymal tissue around the ductus venosus. Mature megakaryocytes or erythroblasts were frequently found in the capillary lumina. However, immature cells of megakaryocytic and erythrocytic series did not increase in the extra-

![Image](image_url)

**Fig. 3.** A late hepatic myeloid progenitor cell stage 1 around the ductus venosus of a human fetus at about 8 weeks of gestation. The cytoplasm contains a moderate amount of single ribosomes and polyribosomes. The chromatin shows central and peripheral clumping. ×13,000
vascular spaces around the ductus venosus. Monocytes or macrophages that contained small granules, bundles of filaments and secondary lysosomes in the cytoplasm were rarely observed there.

Myeloid progenitor cells that showed a lymphoid appearance were first disclosed around the ductus venosus of an embryo about 50 days after ovulation, and then the granulocyte series rapidly increased in number with fetal development.

Mitoses were frequently found in promyelocytes. We could hardly detect mitoses of the late hepatic myeloid progenitor cells, probably indicating that late hepatic myeloid progenitor cells do not undergo mitosis.

Desmosome-like structures, such as reported between the hepatocytes and erythroblasts (Fukuda and Sato, 1971), were not detected between the hepatocytes and the cells of granulocytic lineage.

III. Ultrastructure of the granulocyte series

In this paper, the following nomenclature concerning the granulocyte series is used by referring to previous works (Tanaka and Goodman, 1972; Bessis, 1973; Fukuda, 1974; Cline, 1975).

The adjective "early hepatic" is applied to the immature forms of the granulocyte series which were observed around the ductus venosus at the early stage of hepatic granulopoiesis, and the adjective "late hepatic" to the immature forms of the granulocyte series at the late stage, since the immature cells of granulocytic lineage around the ductus venosus at the early stage differed in ultrastructure from the immature cells

![Fig. 4. A late hepatic myeloid progenitor cell stage II around the ductus venosus of a human fetus at about 8 weeks of gestation. Polyribosomes have increased in number, and the cytoplasm is wider. The chromatin is dispersed with reticular clumps. ×10,600](image)
at the late stage. However, the forms more mature than myeloid progenitor cells did not show any distinct morphological differences between those in the liver before 50 days of ovulation and after 50 days. Therefore, the terms “early hepatic” or “late hepatic” are applied neither to myeloblasts nor to more mature granuloids.

A. Immature cells of the early hepatic granulocytic series

*Early hepatic myeloid progenitor cells* (Fig. 2): These cells, ranging from 11 to 15 μm in diameter (mean: 13 μm), showed a high nucleo-cytoplasmic ratio. The nucleus was indented and had one or two nucleoli. The chromatin was finely dispersed, and the nuclear pores were numerous. The perinuclear cisterna was not dilated. The cytoplasm contained a moderate amount of polyribosomes, a few short slender cisternae of the rough endoplasmic reticulum, a moderate number of mitochondria and a pair of centrioles. The Golgi apparatus was composed of a small number of lamellar cisternae and small vesicles.

B. Immature cells of the late hepatic granulocyte series

These cells were classified into three stages: stage I, II and III.

1. *Late hepatic myeloid progenitor cells stage I* (Fig. 3): These cells were round or oval in shape, and ranged in size from 7 to 9 μm (mean: 8 μm). An indented nucleus occupied most of the cell volume and had a small nucleolus. The chromatin showed moderate clumping. The nuclear pores were small in number. The narrow cytoplasm

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Fig. 5. A late hepatic myeloid progenitor cell stage III around the ductus venosus of a human fetus at about 8 weeks of gestation. Large nucleoli are prominent in the nucleus. Numerous polyribosomes are found in the cytoplasm. Cisternae of the rough endoplasmic reticulum are rarely found. ×10,600
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contained an amount of mono-ribosomes, and polyribosomes, a few small granules, a pair of centrioles and mitochondria. Cisternae of the rough endoplasmic reticulum were hardly ever found. The Golgi apparatus was composed of a few small vesicles and short lamellar cisternae.

2. Late hepatic myeloid progenitor cells stage II (Fig. 4): These cells were oval in shape. The nucleus was more or less deeply cleaved. It contained a small nucleolus. The chromatin showed reticular clumping, and the nuclear pores increased in number. The cytoplasm contained a few small granules, a pair of centrioles and a moderate amount of polyribosomes and single ribosomes. Mitochondria had also increased in number. However, cisternae of the rough endoplasmic reticulum were only rarely found and the Golgi apparatus was small in size.

3. Late hepatic myeloid progenitor cells stage III (Fig. 5): They were irregular in contour being 10-13 μm in the largest diameter (mean: 12 μm). The nucleus was indented in shape, and had a prominent nucleolus. The chromatin was uniformly dispersed in the nucleus, showing more or less reticular clumping. The nuclear pores were further increased in number. The perinuclear cisterna was narrow. The cytoplasm became wider and contained mitochondria, a pair of centrioles and many polyribosomes. Cisternae of the rough endoplasmic reticulum were rare. The Golgi apparatus became larger, and was composed mainly of lamellar cisternae. A few small granules, being 0.1-0.16 μm in diameter (mean, 0.14 μm), were rarely found in the cytoplasm.

Fig. 6. A myeloblast around the ductus venosus of a human fetus at about 8 weeks of gestation. The chromatin is finely dispersed in the nucleus. Slender cisternae of the rough endoplasmic reticulum are increased in the cytoplasm and Golgi apparatus is larger. No primary granule is apparent. ×7,900
C. Myeloblasts and more mature myeloids

1. Myeloblasts (Fig. 6): These cells measured 12-18 µm in diameter (mean: 16 µm). The nucleus contained one or two large nucleoli. The chromatin was finely dispersed in the nucleus, and the nuclear pores were numerous. The perinuclear cisterna was still narrow. Long slender cisternae of the rough endoplasmic reticulum and polyribosomes had increased in number in comparison with the early or late hepatic myeloid progenitor cells, and were distributed diffusely throughout the cytoplasm. The Golgi apparatus became larger, and consisted of lamellar cisternae and small vesicles. A pair of centrioles was present near the Golgi apparatus. A few small granules similar to those of the late hepatic myeloid progenitor cells stage III were often found in the cytoplasm, but primary granules were not observed around the Golgi apparatus. Mitochondria were as many as 14 to 19 per section.

2. Promyelocytes stage I (Fig. 7): These cells were irregular in shape. The nucleus was similar to that of the myeloblasts. The perinuclear cisterna and cisternae of the rough endoplasmic reticulum were slightly dilated. Small vesicles and lamellar cisternae of the Golgi apparatus had increased in number. A small number of primary granules ranging from 0.15 to 0.3 µm in diameter was found to be formed in the lateral dilatation of the Golgi apparatus. Usually a pair of centrioles occurred between the nucleus and the Golgi apparatus. Mitochondria and polyribosomes were quite similar in number and distribution to those in the myeloblasts.

Fig. 7. A promyelocyte stage I around the ductus venosus of a human fetus at about 6 weeks of gestation. The perinuclear cisterna and cisternae of the rough endoplasmic reticulum are slightly dilated. A few small primary granules are found to be formed near the large Golgi apparatus. ×8,800
3. **Promyelocytes stage II** (Fig. 8): The nucleus possessed one or two prominent nucleoli and the chromatin was finely dispersed. Both the perinuclear cisterna and cisternae of the rough endoplasmic reticulum were dilated, and contained an amorphous material. The Golgi apparatus was composed of numerous lamellar cisternae and small vesicles. Primary granules increased in number. These granules measured up to 0.7 μm in diameter. Polyribosomes were markedly decreased.

4. **Promyelocytes stage III** (Fig. 9): These had a reniform nucleus which had a less prominent nucleolus than in stage II. The chromatin was condensed along the inner aspect of the nuclear membrane. The cytoplasm was packed with numerous primary granules. The cisternae of the rough endoplasmic reticulum were less developed, and polyribosomes were less abundant. Mitochondria were few in number. The Golgi apparatus was also smaller.

VI. **Myeloperoxidase of the cells of granulocytic series**

A. **Myeloid progenitor cells** (Fig. 10)

A peroxidase reaction product was often visible in the small granules of late hepatic myeloid progenitor cells. No reaction product was seen in the perinuclear cysterna, Golgi apparatus or cisternae of the rough endoplasmic reticulum.

Fig. 8. A promyelocyte stage II around the ductus venosus of an human fetus at about 9 weeks of gestation. The perinuclear cisterna and cisternae of the rough endoplasmic reticulum are markedly dilated, and contain an amorphous material. The cytoplasm contains large and small primary granules. ×7,900
B. Myeloblasts (Fig. 11)

Peroxidase activity was localized in the Golgi apparatus, cisternae of the rough endoplasmic reticulum, perinuclear cisterna and small granules.

C. Promyelocytes (Fig. 12)

The reaction was confined to the Golgi apparatus, cisternae of the rough endoplasmic reticulum, perinuclear cisterna and primary granules. The primary granules of promyelocytes contained variously sized granules.

DISCUSSION

The neutrophilic granulocyte series have been studied ultrastructurally by many investigators, but the greater part of the studies have been done on the immature cells in acute myelocytic leukemia; only a few electron micrographs are available of non-neoplastic human myeloblasts. The present investigation revealed precise ultrastructure of the granulocyte series in the human embryonic liver.

It became obvious that the granulocyte series were concentrated in the intercellular spaces of mesenchymal cells around the ductus venosus or large arteries in the liver during early fetal development. Since such foci were not found in other organs of the embryo, it was concluded that granulopoiesis is restricted to the above mentioned site during early fetal development.

The cells of megakaryocytic and erythrocytic lineages do not increase in this place, and neither erythropoiesis nor megakaryopoiesis, therefore, seems to advance here. On the other hand, the existence of a small number of mature monocytes indicates that
maturation of the monocytic series, though, does take place, here. The immature cells of the monocytic series in rabbits (Nichols, Bainton and Farquhar, 1971) and humans (Nichols and Bainton, 1973) were examined by electron microscopic and cytochemical techniques. It was proposed that promonocytes could readily be distinguished from promyelocytes by the following morphologic criteria: 1) the granules of promonocytes were fewer and generally smaller, and lacked central crystalline substructure such as seen in the granules of promyelocytes (Bainton, Ullyot and Farquhar, 1971); 2) the cytoplasm of promonocytes contained bundles of filaments; and 3) the nuclei of promonocytes were irregular in contour and were often deeply indented. The lack of a central crystalline substructure in the granules and the appearance of bundles of filaments in the cytoplasm seem to be the most reliable morphological criteria for promonocytes, because of the following reasons: 1) smaller granules resembling those of promonocytes are also present in promyelocytes detected in human bone marrow (Tanaka and Goodman, 1972) and in human embryonic liver (Fukuda, 1974) and 2) the granules are far fewer in immature promyelocytes than in mature promyelocytes in human bone marrow (Scott and Horn, 1970; Bainton, Ullyot and Farquhar, 1971; Ackerman, 1971). The cells shown in Figures 7 and 8 resemble promonocytes in the bone marrow of rabbits or humans. However, they differ from promonocytes in that 1) the bundles of fine filaments are hardly noticeable in the cytoplasm of the cell shown in Figure 7; and 2) the crystalline substructure is present in the granules of the cell shown in Figure 8. From these findings, the cells shown in this paper are judged to be granulocytic.

Fig. 10. A small late hepatic myeloid progenitor cell around the ductus venosus of a human fetus at about 10 weeks of gestation. A peroxidase reaction product is visible only in one small granule. ×14,400
We are convinced that the cells shown in Figures 2-5 were more immature than the myeloblasts because polyribosomes and cisternae of the rough endoplasmic reticulum were less abundant in the cells concerned than in the myeloblasts. Thus, they seem to be more immature than the myeloblasts demonstrated previously in human bone marrow (Capone, Weinreb and Chapman, 1964; Tanaka and Goodman, 1972) or in human embryonic liver (Fukuda, 1974).

There are two major morphological differences between the early hepatic myeloid progenitor cells and the late hepatic myeloid progenitor cells. No distinctive morphological differences could be noticed between myeloblasts and more mature forms of the early and later hepatic granulocyte series, notwithstanding. Firstly, the nuclear chromatin is finely dispersed in the early progenitor cells, whereas it shows moderate central and peripheral clumping in the late progenitor cells. Secondly, the former cells are larger in size than the latter forms.

In a previous paper (Emura, 1978), it was surmised that the granulocyte series were differentiated from small lymphoid cells. However, the present investigation showed that a small number of granuloid cells were present among the mesenchymal cells around the ductus venosus before small lymphoid cells appeared there.

From these findings, it seems logical to draw the following major conclusions. The first is that the precursor cells of the early hepatic granulocyte series morphologically differ from the precursors of late hepatic granulocyte series. The second is that the maturation courses of the early hepatic granulocyte series are different from those of the late hepatic granulocyte series.
The myeloperoxidase reaction is commonly used in establishing cytologic diagnosis of acute leukemia, especially in distinguishing myeloblastic from lymphoblastic leukemia, although the localization of myeloperoxidase in immature granuloids is not yet fully understood.

Recent investigations (De Reiffers et al., 1981; Ogawa and Wada, 1982) showed that a myeloperoxidase reaction product was found in lymphoid cells in cases that were diagnosed as acute lymphoblastic leukemia. In electron microscopy, the reaction was confined to small intracytoplasmic granules, although not detected by routine light microscopy. The patient did not respond to a chemotherapy regimen used for acute lymphoblastic leukemia. The lymphoid cells are similar to our late hepatic myeloid progenitor cells in ultrastructure and localization of myeloperoxidase. The finding of peroxidase reaction in our late hepatic myeloid progenitor cells suggests that it is not catalase, because catalase is identifiable only in an alkaline medium (Breton-Gorius, Coquin and Goichard, 1978). Thus, our results indicate that during the early stage of maturation, the cells of the late hepatic granulocyte series have lymphoid appearance, though they contain myeloperoxidase in small granules. Acute myelocytic leukemia may be erroneously diagnosed as lymphoblastic leukemia if an ultrastructural examination on peroxidase activity is not performed.

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