Acta Medica et Biologica
Vol. 39, No. 2, 59-66, 1991

Morphological Analysis of Human Blood Cell Differentiation, with Special Reference to Stem Cells

Yoshihisa OHNISHI[1] and Iwao EMURA[2]

[1]Department of Pathology (II), Niigata University School of Medicine, Asahimachi 1, Niigata 951, Japan, [2]Pathological Division, Niigata University Hospital

Received April 8, 1991

Summary. **The authors investigated hemopoietic stem cells in human embryonic and fetal liver. Pluripotent hemopoietic stem cells in the human embryonic liver were found to originate from the mesenchymal cells among the hepatocytes, and change their features with the development of the embryo. Ultrastructurally, these presumptive hemopoietic stem cells were classified into four subgroups (Types I-IV). Cells of Type I and Type II were large blastic cells resembling myeloblasts. Erythrocytic, megakaryocytic and granulocytic cell were derived from the presumptive hemopoietic stem Type II cells during the early stage of hepatic hemopoietis. On the other hand, Type IV cells were similar to small lymphocytes. Lymphocytes, together with the granulocytic, megakaryocytic and erythrocytic series, differentiated from the presumptive hemopoietic Type IV stem cells during the late stage of hepatic hemopoiesis. The presumptive hemopoietic Type IV stem cells migrated into the bone marrow.**

The relationship between immature hemopoietic cells and hemopoietic stromal cells seems to play an important role in the maturation of hemopoietic cells.

There has been much debate concerning the development of the human blood stem cell, and its morphological features as well have remained unclear. This paper reviews recent findings on the human blood stem cell, primarily those obtained by our own research group.

Hemopoiesis first appears in the yolk sac, then shifts to the liver and finally to the bone marrow. Erythroblasts from the yolk sac are large in size and synthesize hemoglobin (Hb) Gower 1 and Gower 2. During the early stage of hepatic hemopoiesis, hepatic erythroblasts synthesize Hb-F. In other words, erythroid progenitor cells in the yolk sac differ from hepatic erythroid progenitor cells and synthesize different kinds of Hb. With the development of fetuses, however, Hb-A increases in volume. The switching mechanism from Hb-F to Hb-A is obscure. Dieterlen-Lievre and associates proved that the hepatic hemopoietic stem cell originated from liver tissue, and did not migrate from the yolk sac.[1)] On the contrary, hemopoietic stem cells found in the liver migrate into the bone marrow.[2)]

Therefore hepatic hemopoiesis and the relation between hepatic hemopoiesis and bone marrow hemopoiesis is of critical importance for the understanding of human hemopoiesis. Moreover, it is proposed that cell to cell interaction (hemopoietic inductive microenvironment: HIM) is necessary for the differentiation of hemopoietic stem cells.[3-6)] Therefore, evidence for this proposal was also pursued.

Materials and methods

Over 350 human embryonic and fetal livers were obtained through legal abortion. The youngest embryo was estimated as being at 28 days of gestation.

All materials were studied using light, electron, and immuno-electron microscopical methods to clarify the features of immature granulocytic, erythrocytic and megakaryocytic cells (Table 1). The ultrastructures of presumptive hemopoietic stem cells were surmised from those of the most immature cells of the granulocytic, megakaryocytic and erythrocytic series.

Liver hematopoesis

The epithelial cells of the hepatic diverticulum penetrated the mesenchymal tissue of the septum transversum and underwent frequent mitosis to form the liver. Within the first month, only mature erythrocytes derived from the yolk sac were recognized in

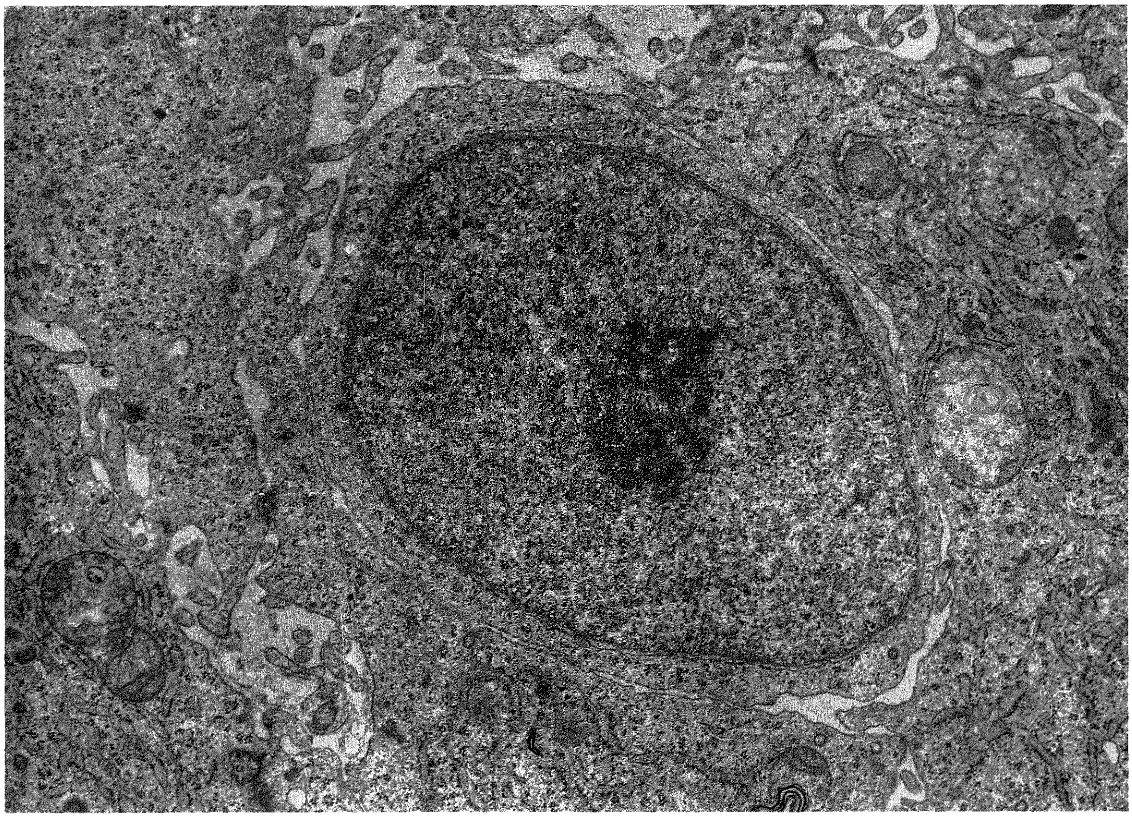


Fig. 1. Presumptive hemopoietic stem cell, Type I. ×10,000. 33rd day in embryonic liver. Large in size, many single ribosomes, euchromatin and small nucleolus.

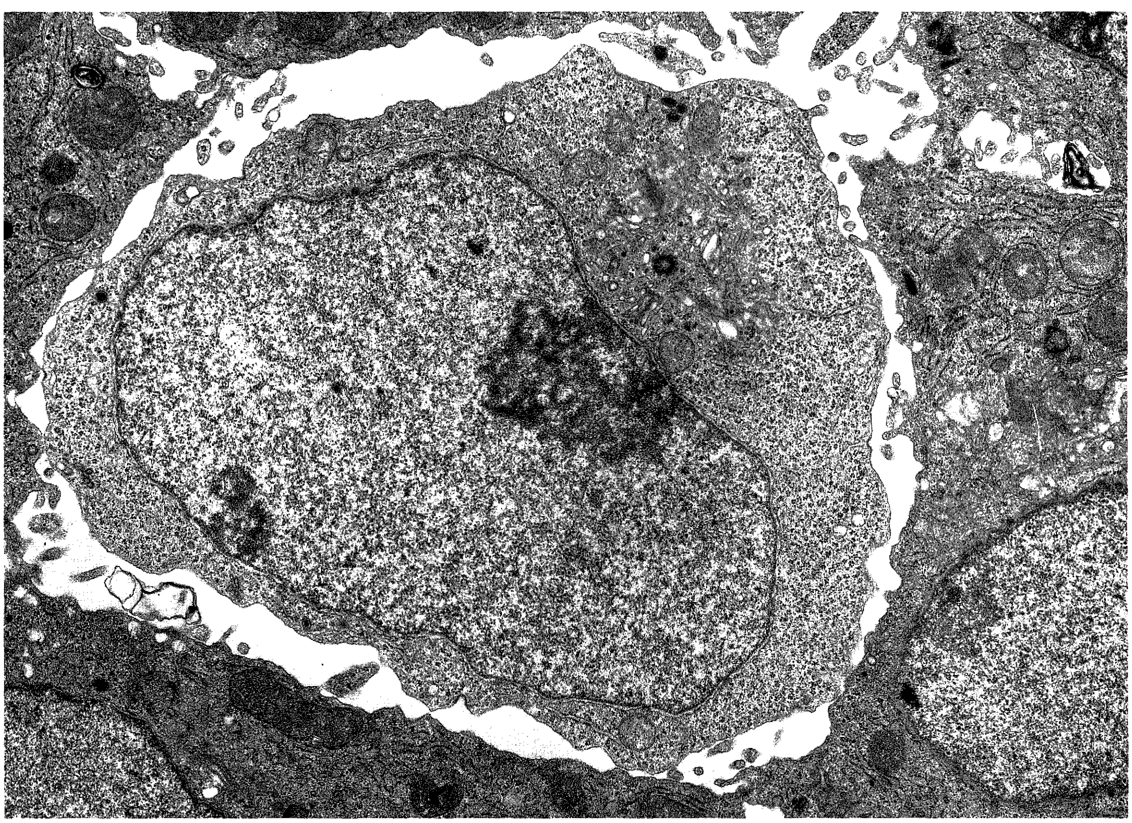


Fig. 2. Presumptive hemopoietic stem cell, Type II. ×10,000. 33rd day in embryonic liver. Large in size, few single ribosome, increaseed polyribosome, euchromatin with large nucleolus.

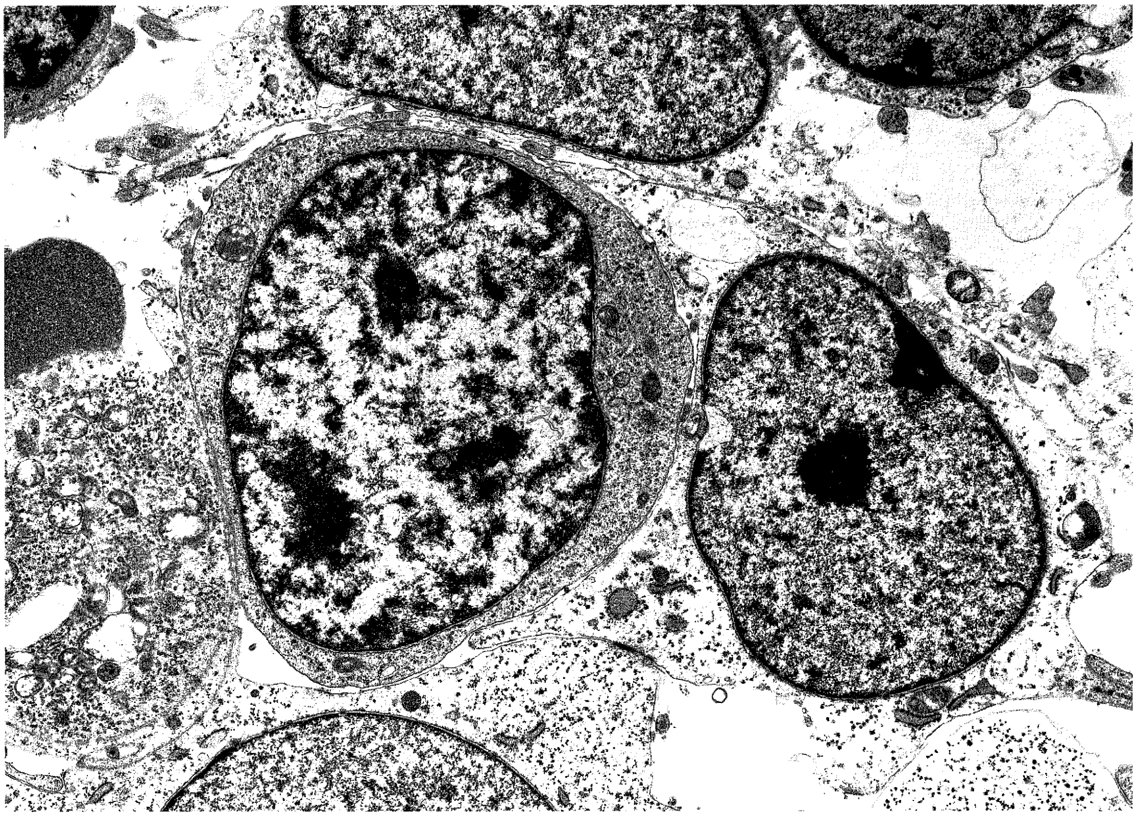


Fig. 3. Presumptive hemopoietic stem cell, Type III. ×10,000. 48th day around the ductus venosum in the embryonic liver.

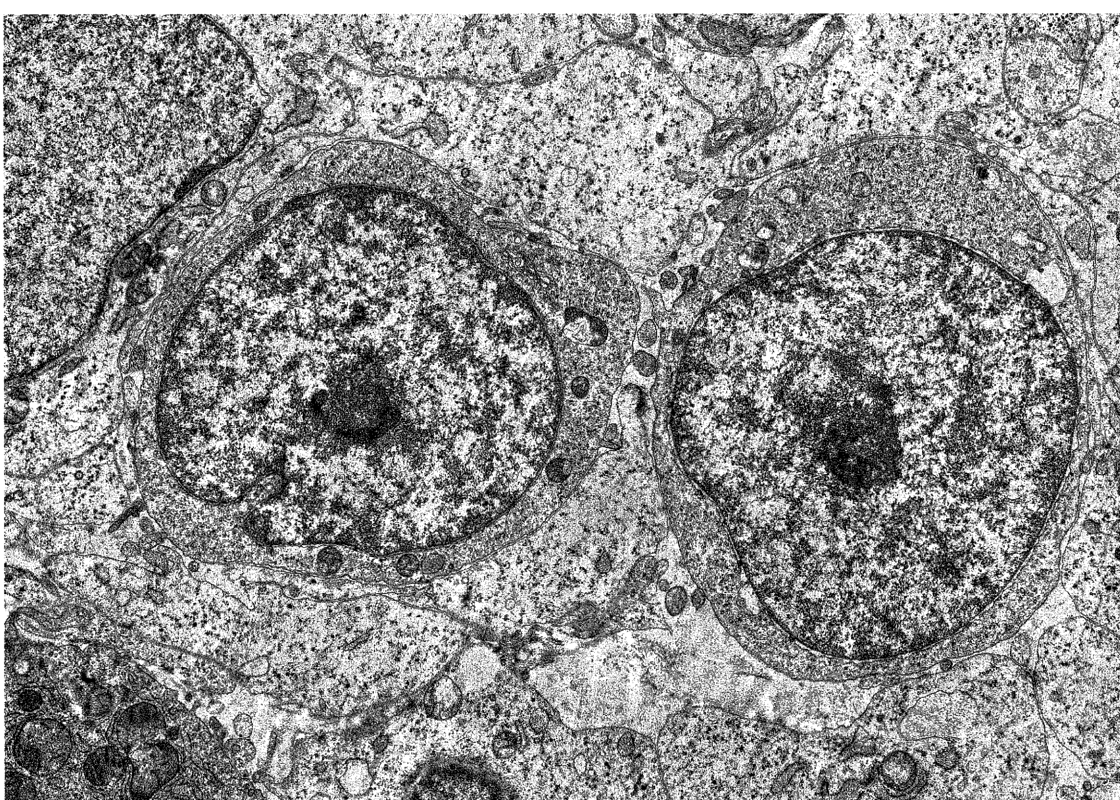


Fig. 4. Presumptive hemopoietic stem cell, Type III. ×7,500. 50th day around the ductus venosum in embryonic liver.

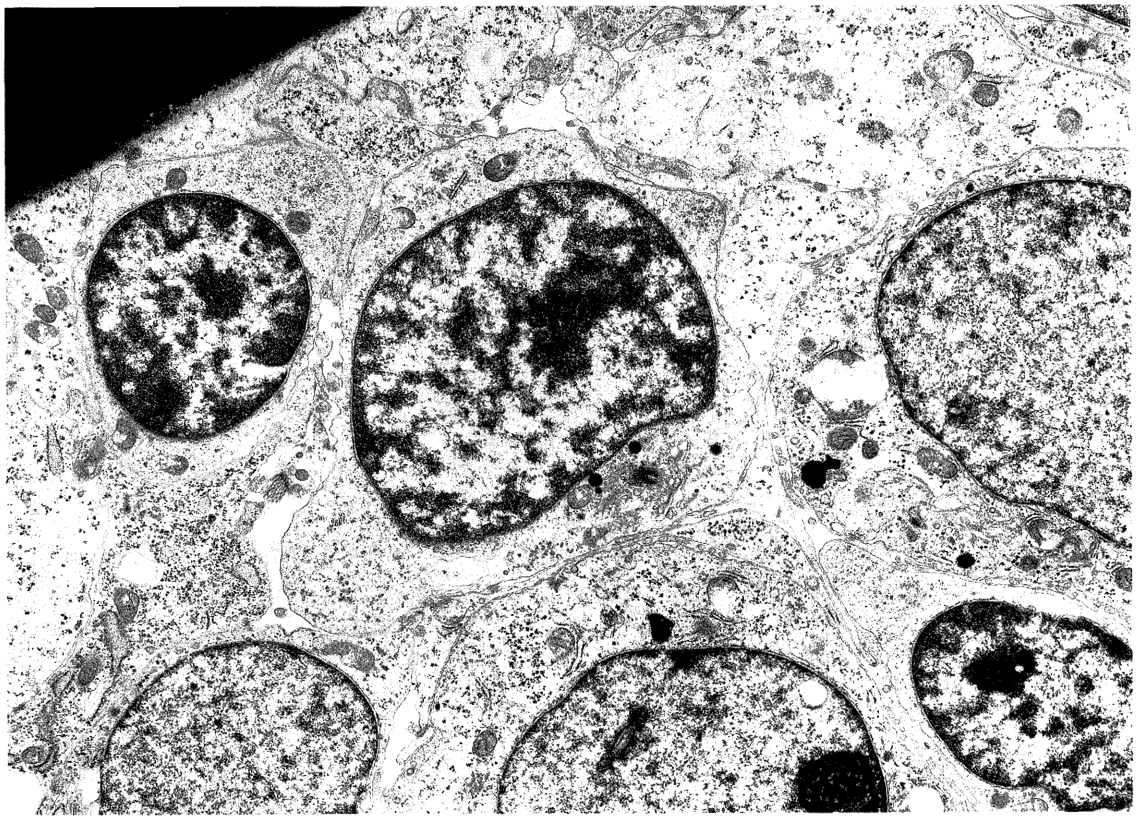


Fig. 5. Presumptive hemopoietic stem cell, Type III. ×7,500. 48th day around the ductus venosum in embryonic liver.

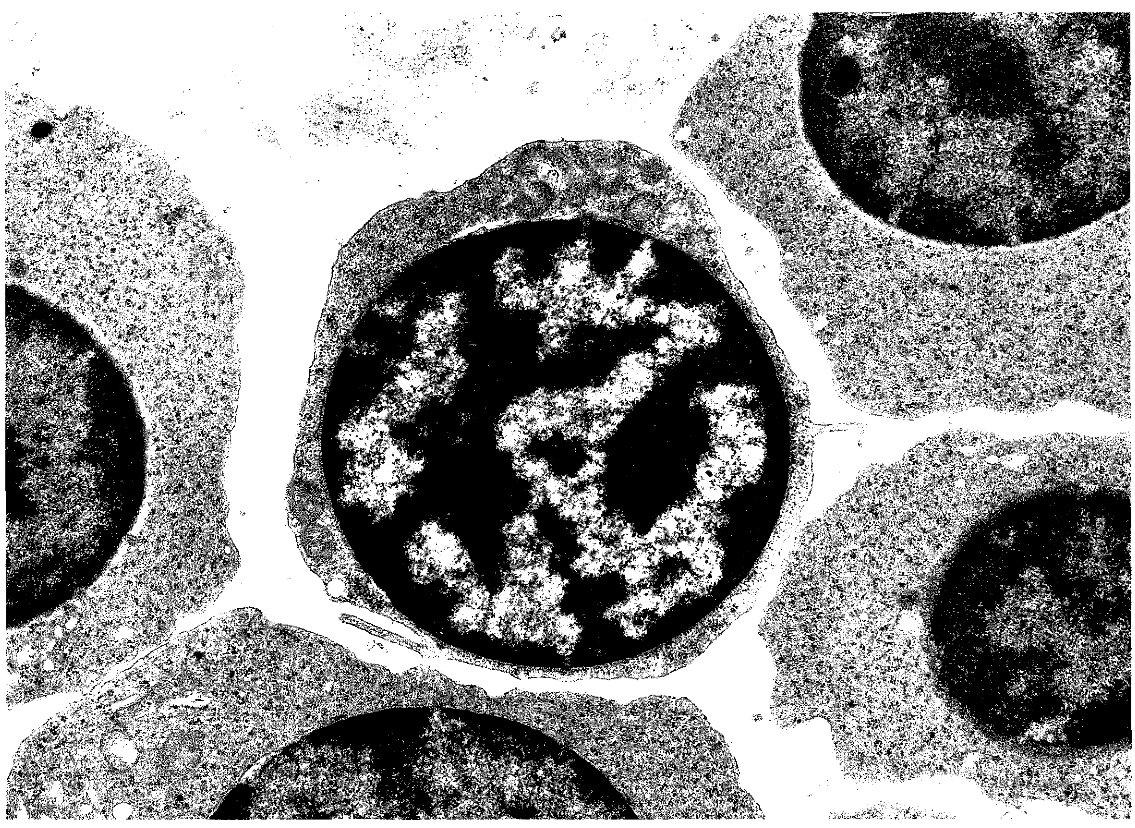


Fig. 6. Presumptive hemopoietic stem cell, Type IV. ×11,000. 45th day in embryonic liver. Note small lymphoid appearance showing heterochromatin in nucleus with few nucleolus.

the blood vessels of the liver. A few undifferentiated blasts (presumptive hemopoietic stem cell Type I: Fig. 1) first appeared among the hepatocytes on the 33rd day following ovulation.[7]

During the following week, both erythropoesis and megakaryopoesis were established in the hepatic parenchyma. Granulopoiesis developed among the reticular cells around the ductus venosus at the 40th day after ovulation. Later, active granulopoesis also developed in the Glisson's sheath. Neither erythropoiesis nor megakaryopoesis developed in Glisson's sheath or in the mesenchymal tissue around the ductus venosus.[7-13]

In the liver, B lymphocytes began to appear from the 60th fetal day and T-lymphocytes appeared from the 75th fetal day.[14]

Bone marrow hematopoesis

Bone marrow hemopoiesis first appeared in the femur during the 11th week after ovulation. A small calcified focus appeared at the center of the cartilaginous rudiments of the vertebrae, and at 14 weeks of fetal age, hemopoiesis began in the vertebrae. At the initial stage of the bone marrow hematopoiesis, only granulocytic cells and a small number of megakaryocytic cells existed in the bone marrow.

Erythropoesis was established three weeks later. Hemopoietic stem cells then migrated into the bone marrow, probably from the liver.[15]

Hematopoietic stem cell

After the experimental study by Till and McCulloch[16] (1961), it was proved that blood cells of all lineages are derived from a multipotential blood stem cell. The ultrastructure of the candidate multipotential hematopoietic stem cells in the bone marrow resemble small or medium sized lymphocytes. We examined the liver, spleen and bone marrow using electron microscopy to clarify the ultrastructure of immature cells of granulocytic, erythrocytic and megakaryocytic lineages.

Table 1. Markers for Each Blast (EM)

Megakaryocytes
Cytoplasmic projection[10]
Demarcation membrane system[10]
Specific granule[10]
Platelete peroxidase[18]
anti glycoprotein IIb IIIa antibody[18,20]
anti platelet antibody (for immunoelectron microscope)[18]
Erythrocytes
Desmosome-like structure[12]
EP1, EP2 (Erythroid progenitor antigen 1, 2)[21]
Granulocytes
Specific granule[7,11]
Myeloperoxidase[10]
Leu M1 for immunoelectronmicroscope[19]

Censequently, we were able to trace the smooth morphological transition from mature to immature cells in each cell lineage. During the early stage of hepatic hematopoiesis (until the 40th fetal day), the most immature cells of all the cell lineages resembled myeloblasts; these cells were regarded to have originated from presumptive hemopoietic Type II stem cells (Fig. 2).

After the 40th day, however, the immature cells of the three hemopoietic series resembling small lymphocytes were found in the liver. These cells were considered to be the derivatives of the presumptive hemopoietic Type IV stem cell (Fig. 6). The presumptive hemopoietic Type III stem cell was the transitional form between the presumptive hemopoietic Type II stem cell and that of Type IV (Figs. 3, 4, 5). Finally the authors classified the presumptive hemopoietic stem cell into four subgroups (presumptive hemopoietic stem cells Types I, II, III and IV).[12,15] The schematic diagram of the blood cell lineages is shown in Tables 2 and 3. The charactrerictics of these presumptive hemopoietic stem cells are listed in Table 4.

Hepatopoietic inductive microenvironment (HIM)

In the embryonic and fetal liver, the relation between immature hemopoietic cells and hemopoietic stromal cells was observed.[13,17]

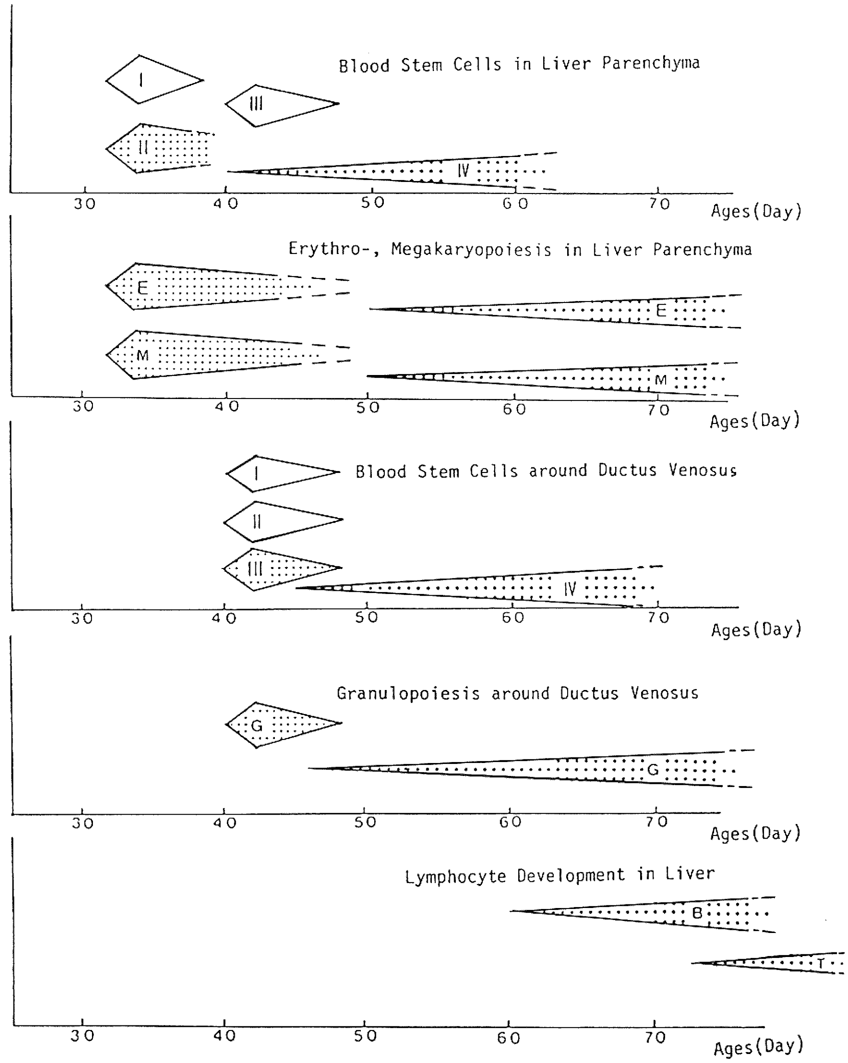
a) Erythrocytic cells

A close relationship is recognized between erythropoiesis and liver cell maturation. Erythropoiesis developed only among mature hepatocytes. Ultrastructurally, immature erythrocytic cells were invaginated in the cytoplasm of hepatocytes, and desmosome-like structures were found between the hepatocytes and erythrocytic cells. Furthermore, slender projections of the hepatocytes were extended into the erythrocytic cells and a pinocytotic pit was formed on the cell membrane of the latter cells.[12]

b) Megakaryocytic cells

Immature megakaryocytic cells were frequently encircled by cytoplasmic projections of reticular cells,

Table 2. Ontogeny of human hemopoietic stem cells and differentiation of blood cells.



E: Erythropoiesis originated from presumptive hemopoietic stem cell type II

M: Megakaryopoiesis originated from presumptive hemopoietic stem cell type II

G: Granulopoiesis originated from presumpitve hemopoietic stem cell type II

E: Erythropoiesis originated from presumptive hemopoietic stem cell type IV

M: Megakaryopoiesis originated from presumptive hemopoietic stem cell type IV

G: Granulopoiesis originated from presumptive hemopoietic stem cell type IV

T: T-lymphocytes originated from presumptive hemopoietic stem cell type IV

B: B-lymphocytes originated from presumptive hemopoietic stem cell type IV

I Presumptive hemopoietic stem cell type I.

II Presumptive hemopoietic stem cell type II.

III Presumptive hemopoietic stem cell type III.

IV Presumptive hemopoietic stem cell type IV.

Table 3. Hemopietic stem cells and differentiation of blood cells.

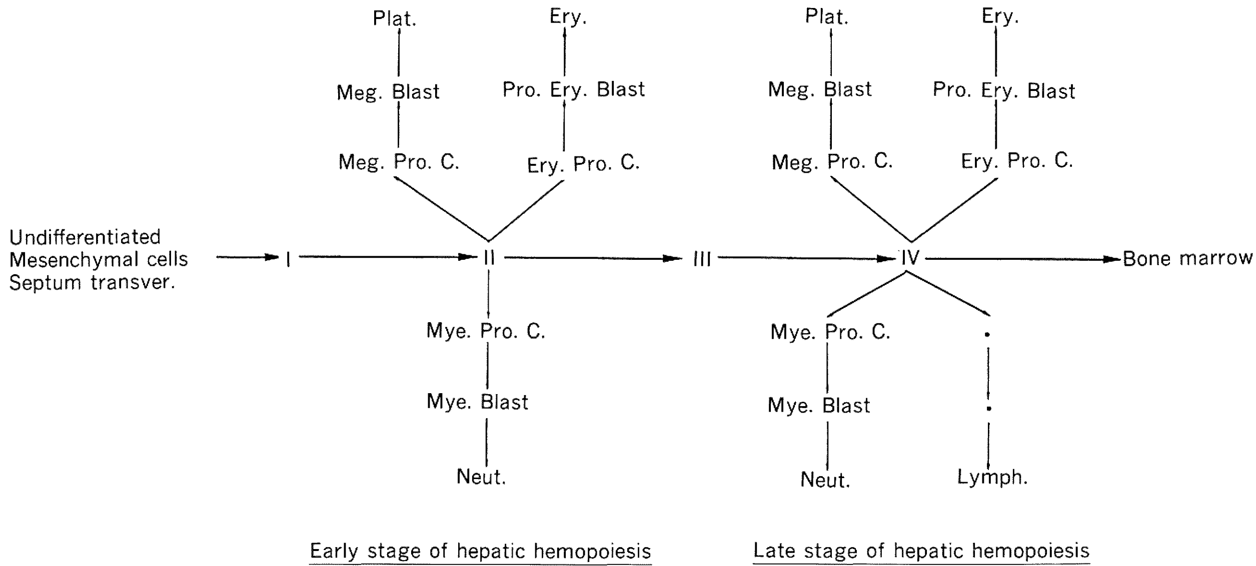
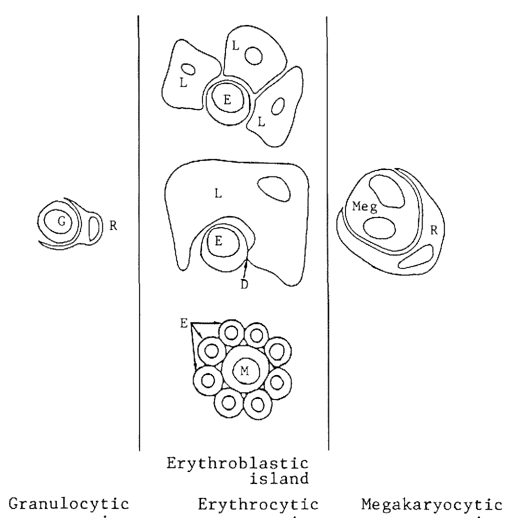


Table 4. Morphological characteristics of hemoietic stem cells

	P.H.S.C. Type I	P.H.S.C. Type II	P.H.S.C. Type III	P.H.S.C. TypeIV
Cells size	9-11	9-15	13-7	6-8
P-ribosome	few	many	many⟶few	few
S-ribosome	few	few	few	few
Chromatin	euchromatin	euchromatin	eu⟶hetero	heterochromatin
Nucleolus	large	large	large⟶small	small

P.H.S.C.: presumptive hemopoietic stem cells
P-ribosome: polyribosome, S-ribosome: single ribosome
eu: euchromatin, hetero: heterochromatin



L: Liver cell, R: Reticular cell
E: Erythroblast, D: Desmozone like structure
R: Granulocytic cell, M: Macrophage
Meg: Megakaryocytic cell

Fig. 7. Schmatic drawings of hemoietic inductive microenvironment.

while reticular cells in small clusters of immature megakaryocytic cells extended their cytoplasmic projections among the megakaryocytic cells.[10] There were no desmosome-like structures between the reticular cells and megakaryocytes.

c) Granulocytic cells

At the beginning of fetal liver hematopoiesis, granulopoiesis appeared around the ductus venosus. After the development of the Glisson's sheath, granulopoiesis was also observed there. Surprisingly, neither erythropoiesis nor megakaryopoesis was found in the Glisson's sheath or around the ductus venosum. We were able to indentify the cytoplasmic projections of reticular cells covering the immature granulocytes and stretching their cytoplasm to each immature granulocyte.[8,16] There was no attachment between reticular cells and immature granulocytes.

The relationship between hemopoietic cells and hemopoietic stromal cells is shown schematically in Fig. 7. On the basis of our findings, we would like to point out that cell to cell interaction plays an important role in the differentiation of hemopoietic cells.

Acknowledgments. We would like express our thanks to Mr. Sato, Mr. Momozaki and Miss Watanabe for their excellent technical assistance.

REFERENCES

1) Dieterlen-Lievre F, Beaupapin D, Martin C: Potentialities and migrations of hemopoietic stem cells of yolk sac and intraembryonic origins, studied in avian chimeras by blastoderm recombination. In: Douarin, le IV (ed) Cell lineage, stem cells and cell differentiation. Worth-holland and publishing company, Amsterdam-New York-Oxford 175-190, 1979.
2) Sekiya M: Human bone marrow development and granulopoiesis in early phase of bone marrow hematopoiesis. *Niigata Med J* **96**: 266-275, 1982. (in Japanese)
3) Curry JL, Trentin JJ: Hemopoietic spleen colony studies. I. Growth and differentiation. *Devel Biol* **15**: 395-413, 1967.
4) Curry JL, Trentin JJ, Cheng V: Hemopoietic spleen colony studies. II. Erythropoiesis. *J Exp Med* **125**: 703-725, 1967.
5) Curry JL, Trentin JJ, Cheng V: Hemopoietic spleen colony studies. III. Hemopoietic nature of spleen colonies induced by lymph node or thmus cells, with or without phytohemagglutinin. *J Immunol* **99**: 907-916, 1967.
6) Curry JL, Trentin JJ: Hemopoietic spleen colony studies. IV. Phytohemagglutinin and hemopoietic regeneration. *J Exp Med* **126**: 819-835, 1967.
7) Emura I: The granulopoesis in the liver of human embryo. *Acta Hematol Jap* **41**: 819-836, 1978. (in Japanese)
8) Ohnishi Y, Emura I: Granulopoesis in fetal liver lymphoid cells and the existence of wrap cells. *Igaku no Ayumi* **105**: 223-224, 1978. (in Japanese)
9) Emura I, Sekiya M, Ohnishi Y: Ultrastructural investigation on human hepatic hemopoiesis. *Recent advances in RES Research* **20**: 11-28, 1980.
10) Emura I, Sekiya M, Ohnishi Y: Two types of immature megakaryocytic series in the human fetal liver. *Arch Histol Jap* **46**: 103-114, 1983.
11) Emura I, Sekiya M, Ohnishi Y: Two types of progenitors of granulocyte series in the human embryonic liver. *Arch Histol Jap* **46**: 229-242, 1983.
12) Emura I, Sekiya M, Ohnishi Y: Two types of immature erythrocytic series in the human fetal liver. *Arch Histol Jap* **46**: 631-643, 1983.
13) Ohnishi Y: Extramedullary hematopoesis—From morphological stand point—. *Sysmex J* **5**: 213-232, 1983. (in Japanese)
14) Abe J: Immunocytochemical characterization of lymphocyte development in human embryonic and fetal liver. *Clinical Immunol Immunopath* **51**: 13-21, 1989.
15) Emura I, Sekiya M, Ohnishi Y: Four types of presumptive hemopoietic stem cells in the human fetal liver. *Arch Histol Jap* **46**: 645-662, 1983.
16) Till JE, McCulloch EA: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Red Res* **14**: 313-322, 1961.
17) Emura I, Sekiya M, Ohnishi Y: Ultrastructural Identification of the hemopoietic inductive microenvironment in the human embryonic liver. *Arch Histol Jap* **47**: 95-112, 1984.
18) Homma K: Morphological study of megakaryocytopoesis in the liver of human embryos and fetuses —immunological approach—. *Acta Hematol Jap* **48**: 1557-1570, 1985. (in Japanese)
19) Takagi A: Immunoelectron microscopical study of the progenitors of the granulocytic series in human embryonal and fetal liver. *Acta Hematol Jap* **50**: 1280-1289, 1987.