Mother Cell of Megakaryocyte*

TERUTOSHI MURATA

*The Second Department of Internal Medicine,† Tohoku
University School of Medicine, Sendai*

MURATA, T. *Mother Cell of Megakaryocyte*. Tohoku J. exp. Med., 1975, 116 (1), 57-66 — It was attempted to describe the morphology of the most immature cell of megakaryocytic series. The megakaryocytes were observed with the electron microscope in five cases, being traced back to their immature forms. In two cases the most immature cells of megakaryocytic series were considered to be the cells which were probably identified as lymphocytes under the light microscope, but they were not lymphocytes with the electron microscope. In other two cases it was presumed that neutrophilic and megakaryocytic series were derived from morphologically similar immature cells, since the most immature cells of neutrophilic and megakaryocytic series were not distinguished when they were traced back to their immature forms. These findings suggest that mother cells of megakaryocytes in the adult bone marrow may be identified as lymphoid cells with the light microscope. — fine structure of megakaryocyte; leukemia; bone marrow

It is widely accepted that megakaryocytes are derived from smaller precursors. The electron microscopic observation on these precursors, that is, mother cells of the megakaryocyte, however, is scanty, although a few articles concerning this problem have appeared (Zamboni 1965; Tanaka and Goodman 1972; Fukuda 1973).

In this paper it was attempted to describe electronmicroscopically the morphology of immature cells in the megakaryocytic series, tracing them back to their most immature forms through the observations of bone marrow in several blood diseases.

MATERIALS AND METHODS

Five cases were chosen for the observation of the bone marrow megakaryocytic series. They had been diagnosed as follows: 1), chronic megakaryocytic leukemia, 2) chronic erythroleukemia (chronic Di Guglielmo syndrome), 3) sideroblastic anemia, 4) May-Hegglin anomaly, and 5) thrombocytosis of undetermined nature. In all of these cases the bone marrow showed a remarkable increase in number of the cells of megakaryocytic series.

Case 1 was thought to be the most important, since it was of a rare type of leukemia and the cells of megakaryocytic series were increased most remarkably in all the cases studied. It should be pointed out that in this case the most immature cells of megakaryocytes identified by electron microscopy closely resembled lymphocytes in their

Received for publication, February 19, 1975.

* Electronmicroscopic studies of human megakaryocytes I.

† Director: Prof. K. Yoshinaga.

light microscopic feature. The detailed clinical findings of this case, as well as of the other cases, were presented in the appendix.

The specimen aspirated from the sternal bone marrow was immediately fixed in chilled 2.5% glutaraldehyde buffered with 0.05 M sodium cacodylate at pH 7.4 for about 1 hr. The specimen was then washed in 0.08 M cacodylate buffer at 4°C for 3 hr or overnight and postfixed in 1% osmium tetroxide in the same buffer. After dehydration in graded acetone, the specimen was embedded in Epon. Ultrathin sections were double stained with uranyl acetate and lead citrate and observed in a Hitachi 7S electron microscope.

RESULTS

Light microscopic findings

The light microscopic observation was mainly performed on Case 1. The leukemic cells could be tentatively classified into three types (type I, II and III) on the smear of May-Grünwald-Giemsa staining.

Type I was a round or oval cell resembling the myeloblast, 10 to 15 μm in diameter. The cytoplasm was narrow and stained pale blue. A small number of azurophilic granules were occasionally seen in the cytoplasm. The granules were distinctly smaller than those of the promyelocyte and just like those of the platelet.

Type II was of the same size as, or slightly larger than, type I. In larger cells cytoplasm was wider than in type I. A few number of lingua-like pseudopodia were always seen protruding from the cytoplasm. In this type azurophilic granules were more abundantly seen than in type I.

Type III had cell diameters between 20 and 40 μm . Lingua-like or larger pseudopodia were always seen and azurophilic granules were often recognized in the pseudopodia. Platelets were occasionally seen aggregating around the leukemic cells. This type seemed to be liberating platelets from the cytoplasm.

These three types showed negative peroxidase reaction by Mac Junkin's method while mature neutrophils were stained strongly positive, and they were considered to be one series and the type I was the most immature. All types of leukemic cells had a round or oval nucleus, sometimes indented. The sizes of these nuclei were 8 to 20 μm in diameter, and chromosome pattern was fine. A few nucleoli were frequently seen. Because it was difficult to differentiate the myeloblast from types I and II, perhaps a small number of myeloblasts might have been included in the leukemic cells. Large mature megakaryocytes were seen very rarely, but the intermediate cells between blast cell and megakaryocyte were abundant on the smear. When the specimen was prepared for the electronmicroscopic observation, type I was 33.4%, type II 12.2% and type III 10.8% of the nucleated cells of the bone marrow, respectively. During his clinical course the three types of leukemic cells in the bone marrow fluctuated with the similar ratio as above and this classification was also possible in the peripheral blood.

Electron microscopic observations

In Case 1 about thirty thousand cells of megakaryocytic series were observed,

Fig. 1a. A leukemic megakaryocyte possessing blebs, bull's-eye granules and microtubules. Case 1. $\times 12,100$.

Fig. 1b. Higher magnification of the microtubules seen in Fig. 1a. $\times 45,100$.

but none of mature megakaryocytes with the demarcation membrane system (DMS) were encountered. The cell shown in Fig. 1a is regarded as a megakaryoblast and possesses the characteristic structures of megakaryocytic series. The granules in the cell, some of which are like bull's-eye, appear to be smaller than those of the promyelocytes. Moderately abundant ribosomes are contained in the cytoplasm. Blebs and microtubules are seen. A platelet is located on the left side of the cell. The cell shown in Fig. 2a has somewhat wider cytoplasm. The cell in this stage possessed numerous mitochondria often more than twenty in number. Golgi apparatus was well developed, occasionally with numerous Golgi vesicles. Sometimes microtubules were seen running parallel with the plasma membrane and near by Golgi apparatus. A small amount of rough surfaced endoplasmic reticulum and granules including bull's-eye granules, were seen. Typical bull's-eye granules are shown in Fig. 2b, which are seen occasionally near the Golgi apparatus. The most immature cell is shown in Fig. 3a. In this stage of maturation, characteristic structures of megakaryocytes such as microtubules, DMS, blebs and bull's-eye granules were not seen. The cytoplasm was narrow. A few to about ten mitochondria were observed. They were small in size and high in density. A few cisternae of rough surfaced endoplasmic reticulum were seen. Golgi apparatus was poorly developed. Granules were not seen usually. Some of these immature cells might be identified as small

Fig. 2a. A leukemic megakaryocyte possessing numerous mitochondria. These mitochondria are small in size and high in density. Case 1. $\times 8,900$.

Fig. 2b. Typical bull's-eye granules near a Golgi apparatus. Case 1. $\times 24,000$.

lymphocytes under the light microscope. In Case 1 the cells of megakaryocytic series possessed round, oval or occasionally indented nuclei, most of which were occupied by euchromatin. Sometimes nucleoli were seen.

In Case 2 mature megakaryocytes were rarely encountered. Most cells of megakaryocytic series, in various stages of maturation, were atypical in form; for instance, small in size, and lacking in granule and DMS. Small lymphoid megakaryoblasts were sometimes seen. Some of immature megakaryocytes possessed microtubules, many mitochondria, blebs and a small number of granules. A few of the granules were like bull's-eye. A less mature cell is shown in Fig. 4a, in which blebs and bulls'-eye granules are seen, but microtubules are not seen. Moderately abundant ribosomes are contained. This cell may perhaps be identified as lymphocyte under the light microscope because of its round and small nucleus and pale blue cytoplasm.

In Case 4 sometimes small megakaryoblasts, as small as myeloblasts, were seen, and the megakaryoblasts had lobulated or indented nuclei and microtubules.

In Case 5 degeneration of mitochondria with myelin-like structures was seen in the cells of megakaryocytic series and often in the plasma cells. Therefore the myelin-like structure in the mitochondria was available as a marker to identify the megakaryocytic cells. The mitochondria were larger than those in the other cases and contained the myelin-like structure as shown in Fig. 5a and b. The most

Fig. 3a. A most immature cell. Characteristic structures for megakaryocytes are not seen in the cell. Case 1. $\times 11,100$.

Fig. 3b. Multivesicular bodies seen in a leukemic megakaryocyte. Case 1. $\times 45,000$.

immature cell in this case, as shown in Fig. 6a and b was identified as the megakaryoblast because of the presence of myelin-like structure in the mitochondria. Characteristic structures for megakaryocytes were not seen. Bull's eye granules and multivesicular bodies were sometimes seen in other cells of megakaryocytic series.

Cases 1, 2 and 5 were suitable for the observation of immature megakaryocytes, since in these cases immature megakaryocytes were markedly increased. In Case 1 the simplest cells, though they did not show characteristic structures of the megakaryocytic series, were considered to be cells belonging to the megakaryocytic series, since the cells of megakaryocytic series were predominant in number compared with other bone marrow components and since there were some numbers of intermediate forms between the simplest cells and megakaryocytes. In light microscopy lymphoid cells constituted 18.6% of the nucleated cells, but lymphocytes were rarely encountered in electron microscopy and most of the lymphoid cells were considered to be the most immature cells of megakaryocytic series.

In Cases 3 and 4 the simplest cells were seen, but they could not be identified as cells belonging to the megakaryocytic series, since the cells of neutrophilic series were predominant and the intermediate forms were not encountered in these cases.

Fig. 4a. A lymphoid megakaryoblast possessing blebs and bull's-eye granules. Case 2. $\times 11,600$.

Fig. 4b. Higher magnification of the bull's-eye granules seen in Fig. 4a. $\times 23,800$.

Through the electronmicroscopic observation of all these cases blebs, bull's eye granules, microtubules and multivesicular bodies (Fig. 3b) were frequently seen in the cells of megakaryocytic series including the immature megakaryocytes.

DISCUSSION

The multipotential hematopoietic stem cell was considered to be the source of megakaryocytes as well as of erythroid and granulocytic cells in some animals and human (Whang et al. 1963). The stem cell is said to resemble the lymphocyte in light microscopic structure by some authors (Cudkowicz et al. 1964; Thomas et al. 1965; Degowin et al. 1971). Dawney and Nordland (1939) described the basophilic lymphoid hemocytoblast and Allegra and Broderick (1971) described the lymphoid megakaryoblast in the human bone marrow, but electron microscopic observation had not been performed by these authors.

It is impossible to identify the stem cell by the reporter's method. But, since the most immature cells of the neutrophilic and megakaryocytic series could not be distinguished when these cells were traced back to their immature forms electron microscopically, it is presumed that the neutrophilic and megakaryocytic series are derived from morphologically the same immature cell. From my observation it is suggested that the most immature cells of megakaryocytic series resemble the

Fig. 5a. Degeneration of mitochondria with myelin-like structure in a megakaryoblast. Case 5. $\times 8,000$.

Fig. 5b. Higher magnification of the myelin-like structure. $\times 27,500$.

lymphocytes light microscopically, but they can not be identified as lymphocytes by the electron microscopy. They resemble the stem cell shown by Zamboni (1965) and Fukuda (1973) in the human embryo and by Van Bekkum et al. (1971) in the mouse bone marrow. These findings are considered to be compatible with the theory that megakaryocytes, as well as erythroid and granulocytic cells, are derived from the lymphoid multipotential stem cells (Till and McCulloch 1961; Nowell and Wilson 1971), but they are distinct from the lymphocytes electronmicroscopically (Van Bekkum et al. 1971).

Blebs, bull's-eye granules, microtubules and multivesicular bodies are often seen in the cells of megakaryocytic series including the immature forms. These organelles, which exist sometimes in other cells, are more frequently observed in the cells of megakaryocytic series than in other bone marrow components. These structures are considered to be useful as markers to distinguish the megakaryocytic series from other bone marrow components. The myelin-like structure seen in Case 5 may be an artifact. Such degeneration was seen only in megakaryocytes and plasma cells, and it is speculated that there are some tendencies to produce easily the myelin-like structure in these two series. Therefore the electronmicroscopic observation of the bone marrow or peripheral blood will contribute much for the diagnosis of megakaryocytic leukemia. It should be stressed, however, that these structures are not seen in the most immature cells of megakaryocytic series.

Fig. 6a. A most immature cell with myelin-like structure. Case 5. $\times 8,500$.

Fig. 6b. Higher magnification of the myelin-like structure. $\times 34,300$.

APPENDIX

Case 1. Chronic megakaryocytic leukemia.

K.Y., a 58-year-old man, a dentist, visited the Ishinomaki Red Cross Hospital in May, 1970, for epigastralgia. Physical examination revealed no pathologic finding except for tenderness of epigastrium, roentgenographic examination revealing gastric ulcer. At this time leukemic cells were found in his peripheral blood unexpectedly.

The laboratory examination revealed that his erythrocyte count was $458 \times 10^4/\text{mm}^3$, hemoglobin content 12.7 g/100 ml, leukocyte count $9,900/\text{mm}^3$ including 1% of leukemic cells. Platelet count was $48.1 \times 10^4/\text{mm}^3$. The bone marrow was hypocellular and contained 19.2% of leukemic cells. At the first time myelogenous leukemia was suspected, but antileukemic drugs were not administered, because the leukemic cells were not abundant and he did not complain of any distress. Leukocyte count and leukemic cell percentage increased gradually. In December 1970, leukocyte count was $15,500/\text{mm}^3$ including 31% of leukemic cells. With the rise of the leukocyte count, erythrocyte count and platelet count decreased gradually.

He had not visited the hospital for 3 months, and when he came again in April, 1971, the liver edge was palpable 8 cm below the right costal margin and the spleen also was 4 cm. Subsequently he was hospitalized. On admission erythrocyte count was $319 \times 10^4/\text{mm}^3$, hemoglobin content 10.5 g/100 ml, leukocyte count $15,900/\text{mm}^3$ including 45% of leukemic cells. Platelet count was $13.1 \times 10^4/\text{mm}^3$. The bone marrow was hypocellular and contained 56.4% of leukemic cells. For the electronmicroscopic observation, the bone marrow was aspirated and subjected for the study.

While he was treated with 100 mg of 6-mercaptopurine and from 30 mg to 40 mg of prednisolone daily, leukemic cells of the peripheral blood and the bone marrow decreased remarkably and the spleen became smaller. But he died of pulmonary infection after 1 month from admission and 1 year from the first visit to the hospital. An autopsy was not performed.

During his clinical course promyelocytes and other immature neutrophils were seen in the peripheral blood with the maximal percentage of 7. These cells, however, were not seen at the incipient stage. Basophils also increased and the maximal percentage was 28. Normoblasts were always seen in the peripheral blood and 32 were detected while 200 leukocytes were counted. Leukocyte alkaline phosphatase score of peripheral blood was 80 or so (normal 170–370). Chromosomal change like Ph¹ chromosome was found in the peripheral blood.

Because some of the leukemic cells closely resembled the myeloblast, myelogenous leukemia was first suspected. Subsequently these leukemic cells were identified as the cells of megakaryocytic series by the electronmicroscopic observation. It was difficult to establish the diagnosis firmly, because autopsy had not been performed and it is the problem of controversy whether megakaryocytic leukemia is a true nasologic entity or not. However, a chronic form of megakaryocytic leukemia was considered as a suitable diagnosis in this case, since immature cells of megakaryocytic series were predominant in the peripheral blood and bone marrow.

Case 2. Chronic erythroleukemia (chronic Di Guglielmo syndrome).

T.K., a 52-year-old man visited the hospital for common cold. Hematologic examination revealed pancytopenia in his peripheral blood and he was admitted. The bone marrow showed erythroid hyperplasia including megaloblastoid cells. At this time leukemic cells were found neither in the peripheral blood nor in the bone marrow. After 8 months the peripheral blood and bone marrow showed leukemic change. He died of cerebral hemorrhage after 13 months from the first visit to the hospital. The specimen used for electronmicroscopic observation was prepared at the time of the admission.

Case 3. Sideroblastic anemia.

N.A., a 23-year-old woman visited our hospital for severe anemia. A marked increase of sideroblasts including ringed sideroblasts was revealed in the bone marrow. She was treated with pyridoxine and folic acid for 18 months, but no response occurred. Then she was treated with pyridoxine and ascorbic acid, and after 14 months her anemia completely healed. The specimen used for electron microscopic observation was prepared before recovery of the anemia. This case will be reported in detail elsewhere.

Case 4. May-Hegglin anomaly.

M.O., a 17-year-old man visited our hospital for recurrent epistaxis. His platelet count was $1.7 \times 10^4/\text{mm}^3$. Giant platelets and Döhle bodies in the neutrophils were disclosed on the smear of peripheral blood.

Case 5. Thrombocytosis of undetermined nature.

T.K., a 24-year-old well nourished woman came to our hospital for migraine-like headache in December, 1972. Her platelet count was markedly increased, the maximum of which was $392 \times 10^4/\text{mm}^3$. She had no sign of bleeding or thrombosis. The liver and spleen were not palpable. Platelet survival time was within the normal range. By electron microscopy degeneration of mitochondria with myelin-like structures was found in the cells of megakaryocytic series and often in the plasma cells. In October, 1973 sternal and iliac bone marrow aspiration resulted in a dry tap, and roentgenographic examination suggested the existence of osteosclerosis. The definite diagnosis on this case, however, has not been established as yet.

Acknowledgment

I am deeply indebted to Prof. Kaoru Yoshinaga, Prof. Tatsuo Torikai, Dr. Seiju Onodera, Dr. Kosuke Oikawa, Dr. Takashiro Suzuki and Miss Noriko Komura of our department for their kind help in performing this study. Furthermore, I wish to thank Dr. Akira Shibata and Dr. Akira B. Miura, Akita University School of Medicine, and Dr. Masaharu Uchimi and Dr. Chikara Suzuki, the Ishinomaki Red Cross Hospital.

References

- 1) Allegra, S.R. & Broderick, P.A. (1971) Acute aleukemic megakaryocytic leukemia: Report of a case. *Amer. J. clin. Path.*, **55**, 197-205.
 - 2) Cudkowicz, G., Bennett, M. & Shearer, G.M. (1964) Pluripotent stem cell function of the mouse marrow "lymphocyte". *Science*, **144**, 866-868.
 - 3) Dawney, H. & Nordland, M. (1939) Hematologic and histologic study of a case of myeloid megakaryocytic hepato-splenomegaly. *Folia haemat.*, **62**, 1-39.
 - 4) Degowin, R.L., Hoak, J.C. & Miller, S.H. (1971) Ultrastructural features of the early proliferating cells involved in hemopoietic colony formation. *Radiat. Res.*, **48**, 495-507.
 - 5) Fukuda, T. (1973) Ultrastructure of human fetal hemopoietic cells presumptive hematopoietic stem cell. *Saishin Igaku* (Jap.), **28**, 1673-1686.
 - 6) Nowell, P.C. & Wilson, D.B. (1971) Lymphocytes and hemic stem cells. *Amer. J. clin. Path.*, **65**, 641-650.
 - 7) Tanaka, Y. & Goodman, J.R. (1972) *Electron Microscopy of Human Blood Cells*. Harper and Row, New York, p. 183.
 - 8) Thomas, E.D., Flidner, T.M., Thomas, D. & Cronkite, E.P. (1965) The problem of the stem cell: Observations in dog following nitrogen mustard. *J. Lab. clin. Med.*, **65**, 794-803.
 - 9) Till, J.E. & McCulloch, E.A. (1961) Direct measurement of radiation sensitivity of normal bone marrow cells. *Radiat. Res.*, **14**, 213-222.
 - 10) Van Bekkum, D.W., Van Noord, M.J., Maat, B. & Dicke, K.A. (1971) Attempts at identification of hemopoietic stem cell in mouse. *Blood*, **38**, 547-558.
 - 11) Whang, J., Frei, E., 3rd, Tjio, J.G., Carbone, P.P. & Brecher, G. (1963) The distribution of the Philadelphia chromosome in patients with chronic myelogenous leukemia. *Blood*, **22**, 664-673.
 - 12) Zamboni, L. (1965) Electron microscopic studies of blood embryogenesis in humans. II. The hematopoietic activity in the fetal liver. *J. Ultrastruct. Res.*, **12**, 525-541.
-