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The Size of Megakaryocytes in Human Fetal, Infantile and Adult Hematopoiesis*)

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ABSTRACT

It has been reported that the appearance of small megakaryocytes can be detected in various blood diseases and in fetal hematopoiesis.

In the studies reported herein, we have investigated the age changes in the area of megakaryocytes during fetal stage to the newborn infant, infant, and adult stage.

The megakaryocyte increased in size from ago of 5 months to 10 months in fetal stage, and then at the age of one year after birth reached a size almost identical to that of adults.

In comparing the megakaryocyte area by fetal age, however, the significant difference was not observed in the hepatic and the bone marrow hematopoiesis at the age of 5 and 7 months.

The results in comparing the area ratio of the nucleus to the cytoplasm of megakaryocytes indicated that the nucleus of fetel megakaryocytes is generally smaller than that of adult marrow megakaryocytes.

This finding suggests that the size of the nucleus of megakaryocytes enlarges with increase in the area of megakaryocytes.

It is also considered that the functional or morphological changes of megakaryocytes may reflect the function or morphology of the platelet.

INTRODUCTION

It is well known that fetal hematopoiesis commences from the yolk sac and then hepatic hematopoiesis becomes predominant in the early to the midterm of pregnancy. From the fifth month of fetal stage, hepatic hematopoiesis gradually decreases to be replaced by the increase in fetal marrow hematopoiesis[1,7,13)]. We have previously reported on our comparison of cell diameter of megakaryocytes during fetal hepatic hematopoiesis and that of adult marrow megakaryocytes, in which it was observed that megakaryocytes seen in the fetal liver are generally smaller than those of the adult bone marrow[4)].

This report describes the age changes in megakaryocyte area from the fetal stage to the newborn infant, infant, and adult stages.

MATERIALS AND METHODS

Examination was made on megakaryocytes of the liver (fetal stage) and the bone marrow (fetal, infant and adult stages). All specimens were obtained from autopsy materials (7 cases of 5 months, 4 cases of 7 months, 2 cases of 10 months). Most of the fetal materials (13 cases) were cases of artificial abortion. The cause of death of 4 newborn infants (less than 1 year), 4 infants (1-4 years), and 2 adults were not blood disease. After fixing the fetal liver in formalin, histological specimens were routinely prepared and then stained with hematoxylin, eosin or PAS. As for bone marrow specimens,

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after fixing in formalin and decalcification, they were embedded in paraffin according to standard procedure and thin specimens thus obtained were stained with hematoxylin, eosin or PAS. The mature megakaryocytes in the specimens were observed at 1,000 power magnification and photographed. The photographic negatives were enlarged at a fixed enlargement rate and the megakaryocyte area was measured by a mortion analyzer (NAC Company).

RESULTS

Table 1 shows a comparison of the area of megakaryocytes observed in the fetal liver and bone marrow. The results are the means of the measured values.

The mean area of hepatic megakaryocytes of 5 months old fetuses was $11.3 \pm 4.3\,cm^2$ and that of marrow megakaryocytes of the same age was $10.5 \pm 3.0\,cm^2$. The mean area of hepatic megakaryocytes of 7 months old fetuses was $13.8 \pm 4.3\,cm^2$ and that of marrow megakaryocytes of the same age was $14.5 \pm 4.8\,cm^2$. There was no significant difference between fetal hepatic megakaryocytes and fetal marrow megakaryocytes of the same age. However, the mean area of megakaryocytes of 7 months old fetuses was significantly larger than that of 5 months old fetuses.

The changes in the megakaryocyte area with fetal age and age after birth are shown in Table 2 and Fig. 1. As shown in Table 2, the mean area of megakaryocytes was $10.8 \pm 3.2\,cm^2$ at

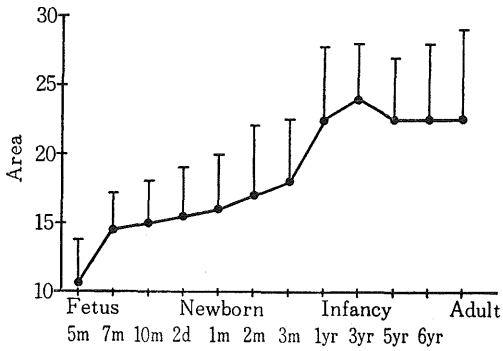

Fig. 1. Change in area of megakaryocytes from fetal stage to adulthood

Table 1. Area of megakaryocytes in human fetal liver and bone marrow and in adult bone marrow

	Number of cases	Number of cells examined	Area of cells (Actual measurement cm^2)*
Fetal 5 months			
Liver	2	49	11.3±4.3
Bone marrow	5	81	10.5±3.0
Fetal 7 months			
Liver	2	59	13.8±4.3
Bone marrow	2	65	14.5±4.8
Adult bone marrow	2	40	22.6±6.2

* True area = Actual measurement $\times \frac{1}{625} \times 10^4\,\mu m^2$.

Table 2. Marrow megakaryocyte area during fetal, neonatal and infancy stages

Materials	Number of cases	Number of cells examined	Area of cells (Actual measurement cm^2)*
Fetal (5 months)	7	130	10.8±3.2
(7 months)	4	122	14.0±4.2
(10 months)	2	65	14.9±2.9
Newborn (<1 year)	4	83	16.8±4.1
Infancy (1-6 years)	4	74	23.0±4.7
Adult	2	40	22.6±6.2

* True area = Actual measurement $\times \frac{1}{625} \times 10^4\,\mu m^2$.

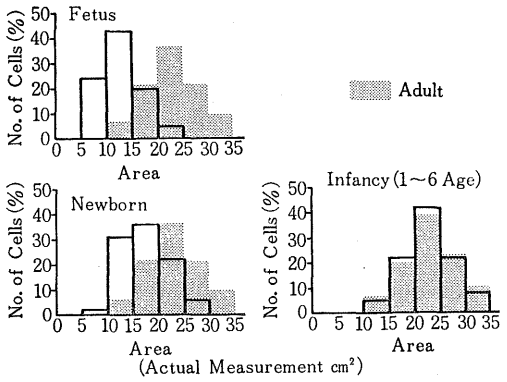

Fig. 2. Distribution of megakaryocyte area of fetal, neonatal, infantile and adult bone marrow

5 months, 14.0±4.2 cm² at 7 months, 14.9±2.9 cm² at 10 months, 23.0±4.7 cm² at less than 1 year after birth, 23.0±4.7 cm² at 1-6 years, and 22.6±6.2 cm² in adults.

The mean megakaryocyte area increased with fetal age, however, the megakaryocytes of 10 months old fetuses were smaller than that of adult marrow megakaryocytes. Furthermore, in the cases less than 1year after birth (2 days, 24 days, 2 months, 3 months), the megakaryocyte area were smaller than those of adults. Fig. 2 shows the distribution of the area of megakaryocytes of those from age 1 to 6 years using as standard the distribution of the area of adult marrow megakaryocytes. The mean area of marrow megakaryocytes of cases older than 1 year (1 year: 22.6±5.1 cm², 3 years: 24.2±3.8 cm², 5 years: 22.3±4.7 cm², 6 years: 22.5±5.5 cm²) shows no difference from that of adults. As evident from Fig. 2, there were many small megakaryocytes during the fetal hematopoietic stage when compared to those of infants less than 1 year of age. Both the distribution of megakaryocyte area during the fetal stage and the distribution of megakaryocytes by area in infants less than 1 year of age did not show a normal distribution. However, the distribution of area of megakaryocytes of the age of 1 to 6 years showed almost the same tendency as that of adults. The distribution of marrow megakaryocytes of infants and adults both showed a normal distribution.

Table 3 shows the area ratio of nucleus to cytoplasm of megakaryocytes. The area ratio of the nucleus to the cytoplasm during the fetal hematopoietic stage was 19.2%, whereas that

Table 3. Area ratio of nucleus to cytoplasm of marrow megakaryocytes

	Nucleus/Cytoplasm (%)
Fetus (Liver, Bone marrow)	19.2
Adult (Bone marrow)	20.1

in adults was 20.1%. The area ratio of the nucleus to the cytoplasm in megakaryocytes did not show any significant diffecence between the fetal stage and the adult stage.

DISCUSSION

It has been reported that the appearance of small megakaryocytes in various blood diseases can be detected[2,3,6,10-12]. We have also reported previously that small megakaryocytes can be observed in hepatic hematopoiesis of the normal human fetus[4]. With regard to the question whether the procedure of decalcification has any effect on marrow megakaryocyte area, we have already observed no difference between in megakaryocyte diameter in clot section obtained by aspiration of adult bone marrow without the decalcification and in bone marrow tissue specimen following decalcification[4]. Therefore, it is considered that after fixing with formalin there is no large difference in cell size with or without the decalcification. In comparing the megakaryocyte area by fetal age, no significant difference was observed in the liver and bone marrow at the age of 5 and 7 months. This indicates that no difference can be observed by site of hematopoiesis in comparing the mean area of megakaryocytes in the fetal stage.

In studying the changes in mean area of megakaryocytes by fetal age, it was confirmed that megakaryocytes increased in size from the fetal age of 5 months to that of the fetal age of 7 months and to that of the fetal age of 10 months and then the mean area of marrow megakaryocytes at the age of 1 year after birth reached a size almost identical to that of adults. At the age of one year after birth the distribution of the area of marrow megakaryocytes also showed no difference from that of adults.

The results in comparing the area ratio of the nucleus to the cytoplasm of megakaryocytes indicated that the nucleus of fetal megakaryocytes is generally smaller than that of adult marrow megakaryocytes. This finding suggests that the size of the nucleus enlarges with in-

crease in the area of megakaryocytes. According to Nakeff et al.[8], with augment in nucleic DNA volume the cell size increases and with enlargement in nucleus size ploidy increases. In estimating the distribution of megakaryocyte ploidy obtained from the present study, during the fetal stage and neonatal stage megakaryocytes having fewer ploidy are more predominant than in adults. At the age of one year after birth, however, it is considered that ploidy distribution of megakaryocytes becomes similar to that of adult marrow megakaryocytes from the age of one after birth.

Penington et al.[9] reported that adult marrow megakaryocytes mature until the stage of the cytoplasmic disintegration and the nucleic DNA volume at such time shows three levels, that is, 8n, 16n and 32n. At each ploidy class specific granules and demarcation membrane are observed, respectively. Megakaryocytes with 16n ploidy are most predominant, occupying 67% of adult bone marrow megakaryocytes. Harker[5] has also reported that megakaryocyte volume is correlated to each class of ploidy.

We have previously reported by using the electron microscopy that the imperfect formation of demarcation membrane, the fewer distribution of specific granules, and the differentiation asynchronism of the nucleus and cytoplasm (the poor formation of demarcation membrane within the cytoplasm to compare the maturity and the differentiation of nuclei) were seen predominantly in the megakaryocytes of human fetuses[4]. It is also considered that the functional or morphological changes of megakaryocytes may reflect the function or morphology of the platelet.

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REFERENCES

1. **Bloom, W.** 1938. Embryogenesis of mammalian blood, p. 863-922. *In* H. Downey (ed.), Handbook of Hematolgy, vol. 2, Hafner Publ. Comp., New York.
2. **Breton-Gorius, J., Drefus, B., Sultan, C., Basch, A. and D Oliveria, J. G.** 1972. Identification of circulating micromegakaryocytes in a case of refractory anemia. An electron microscopic-cytochemical study. Blood **40** : 453-463.
3. **Breton-Gorius,J. and Guichard, J.** 1972. Ultrastructural localization of peroxidase activity in human platelets and megakaryocytes. Am. J. Pathol. **66** : 277-286.
4. **Enzan, H., Takahashi, H., Kawakami, M., Yamashita, S., Ohkita, T. and Yamamoto, M.** 1980. Light and electron observations of hepatic hematopoiesis of human fetuses. II. Megakaryocytopoiesis. Acta Pathl. Jpn. **30** : 937-954.
5. **Harker, L. A. and Finch, C. A.** 1969. Thrombokinetics in man. J. Clin. Invest. **48** : 963-974.
6. **Maldonado, J. E.** 1974. Dysplastic platelets and circulating megakaryocytes in chronic myeloproliferative diseases. II. Ultrastructure of circulating megakaryocytes. Blood **43** : 811-820.
7. **Maximow, A.** 1927. Die embryonale Entwicklung des Blutes und Bindegewebes. 1. Saugetiere, p. 466-517. In Handbuch der mikroskopischen Anatomie des Menschen, vol. 2, pt. 1, Springer, Berlin.
8. **Nakeff, A., Valeriote, F., Gray, J. W. and Grabske, R. J.** 1979. Application of flow cytometry and cell sorting to megakaryocytopolesis. Blood **53** : 732-745.
9. **Penington, D. G.,** Streatfield, K. and Roxburgh, A. E. 1976. Megakaryocytes and the heterogeneity of circulating platelets. Brit. J. Haemat. **34** : 639-653.
10. **Smith, W. B., Ablin, A., Goodman, J. R. and Brecher, G.** 1973. Atypical megakaryocytes in preleukemic phase of acute myeloid leukemia. Blood **42** : 535-549.
11. **Thiele, J., Holgado, S., Choritz, H. and Georgii, A.** 1982. Abnormalities of megakaryocytes in myelitis and chronic myeloproliferative diseases. Virchows Arch. [Cell Pathol] **41** : 67-82.
12. **Wiesneth, M., Pflieger, H., Kubanek, B. and Heimpel, H.** 1980. Micromegakaryocytes in human bone marrow. Acta haemat. **64** : 65-71.
13. **Wintrobe, M. M.** 1974. Blood formation in the embryo and fetus, p. 53-55. In Clinical Hematology, 7th ed. Lea & Febiger, Philadelphia.