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Morphometric Studies of Megakaryocytes in Human and Rat Fetal, Infantile and Adult Hematopoiesis
I. Observations on human fetuses and blood dyscrasias

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ABSTRACT

As a basic study of the thrombocytic productivity of megakaryocytes. Author have investigated the megakaryocyte size from fetal stage to the adult stages.

In the present study, a comparative review was made on the megakaryocyte size in blood dyscrasias (AML, ALL and ITP). In comparison with the mean megakaryocyte size of adult used as control, the mean size in AML and ALL was smaller, but the mean size in ITP was larger than the control.

The megakaryocyte size during fetal hematopoietic stage was remarkably smaller than that in acute leukemia.

Further data were added to author findings reported previously on the megakaryocyte size during the fetal, neonatal, infantile and adult stages of man, but these additional data did not bring about hardly any change to the results of author previous report.

1) There was no significant difference between fetal hepatic megakaryocytes and fetal marrow megakaryocytes of the same age.

2) The mean megakaryocyte size during the fetal hematopietic stage increased with lapse of time.

3) The mean megakaryocyte size after birth increased from the fetal stage for at least to one year after birth when they became almost equivalent in size to those of adults.

In hematopoiesis in the fetal stage of man, the site of hematopoiesis changes with differentiation. Hematopoiesis begins in the yolk sac in the initial stage of gestation, and then the fetal liver becomes the predominant hematopoietic organ in from early to midterm gestation. Hematopoiesis in the liver gradually decreases and hematopoiesis in the bone marrow increases in its place from around the fifth month of fetal age[2,11,21]. Hematopoiesis in the bone marrow becomes greater than that in the liver from around the seventh month of fetal age to account for the greater part of the prenatal hematopoiesis. Megakaryocytes, which are thrombocyte-producing mother cells, derive from stem cells. Megakaryocytes are classified by their morphology into megakaryoblasts, promegakaryocytes and mature megakaryocytes. Megakaryoblasts have a large-sized nucleus, and their cytoplasm is basophilic and presents no granules. Granules

are abundantly found in mature megakaryocytes.

Presence of a relationship of the thrombocytic productivity of megakaryocytes to the number and size of the cells have been demonstrated[6,13–15,17], and it has been reported from a comparison of the diameters of megakaryocytes of the human fetus in the stage of hepatic hematopoiesis with the diameters of adult bone marrow megakaryocytes that the size of the former is generally smaller than the size of the latter[7,8], As a basic study of the thrombocytic productivity of megakaryocytes, I have made for the prenatal stage, neonatal stage and adult stage with the addition of new data an examination of the changes attributable to age and also a comparative study with the addition of AML, ALL, and ITP as blood dyscrasias. The results will be reported.

MATERIALS AND METHODS

All the specimens examined were obtained from autopsy materials. Eighteen cases were fetal, 5 neonatal, 5 infant, and 4 adult, and none of them were cases of blood dyscrasias. As cases of blood dyscrasias, 11 cases of AML, 14 of ALL, and 3 of ITP were used. Histological specimens of the fetal liver were routinely prepared after fixation in formalin, and then stained with hematoxylin or eosin. All bone marrow specimens were prepared by the conventional procedure following fixation in formalin and decalcification; i.e., imbedded in paraffin, cut in thin sections, and stained with hematoxylin or eosin. Mature megakaryocytes in the specimens were observed under 1,000 power magnification and photographed. Enlarged prints were prepared from the films at a fixed enlargement rate, and megakaryocytes size was measured using a mortion analyzer (NAC Company).

RESULTS

1) Comparison between liver and bone marrow

A total of 34 cases, 20 fetal and 14 infantile and adult were studied, and 28 cases were studied in relation to blood dyscrasias. A comparison of fetal megakaryocyte size between the liver and bone marrow is shown in Table 1. The results are shown as means of observed values, that is, values (relative values) determined on the enlarged prints. These results show no significant difference in the mean megakaryocytes size as compared between megakaryocytes in fetal hepatic hematopoiesis and the same in bone marrow hematopoiesis at the same fetal age.

2) Observed results with lapse of time

Observing the changes in the size of megakaryocytes with time, we found the mean megakaryocyte size to be significantly larger at 7 months of fetal age than at 5 months. The

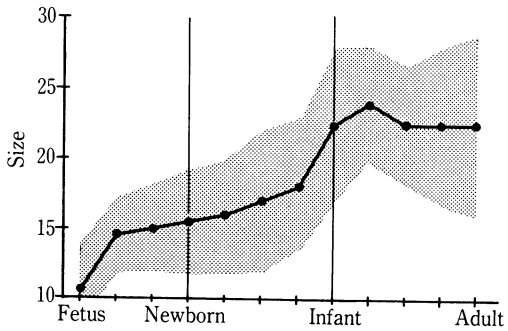

Fig. 1. Change in size of megakaryocytes from fetal stage to adulthood

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

	Number of cases	Number of megakaryocytes	Cell size (Actual measurement cm^{2*})	(True cell size) μm^2
Fetus 5 months				
Liver	4	80	11.0 ± 2.9	(176.0)
Bone marrow	6	101	10.9 ± 3.4	(174.4)
Fetus 7 months				
Liver	4	102	13.7 ± 4.0	(219.2)
Bone marrow	4	100	14.2 ± 4.3	(227.2)
Adult bone marrow	4	100	22.7 ± 5.3	(363.2)

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

Table 2. Size of megakaryocytes during fetal, neonatal and infancy stages

Materials	Number of cases	Number of megakaryocytes	Cell size (Actual measurement cm^{2*})	(True cell size) μm^2
Fetus (5 months)	10	181	10.9 ± 3.2	(174.4)
(7 months)	8	202	14.0 ± 4.1	(224.0)
(10 months)	2	65	14.9 ± 2.9	(238.4)
Newborn (<1 year)	5	107	16.9 ± 3.9	(270.4)
Infant (1-6 years)	5	101	23.0 ± 4.4	(368.0)

changes in mean megakaryocyte size occurring with progression of fetal age or age are shown in Table 2 and Fig. 1. As evident in Fig. 1, the mean megakaryocyte size was significantly larger at 7 months of fetal age than at 5 months. Postnatally, with the cases divided into those under one year of age, those one year or over and under six, and those who were adults, the sizes of those under age one (newborns) were somewhat smaller compared with the sizes in those one or over and under six years of age and the adults. However, there was no evident difference in mean megakaryocyte size between infants and adults.

3) Comparison of megakaryocyte size and distribution

Fig. 2 compares the distributions of megakaryocyte sizes individually, in fetuses, newborns, infants, and adults. As compared to megakaryocytes of adults, only 9, or about 3%, of the megakaryocytes in the fetal hematopoietic stage were of the same size as the average megakaryocytes of adults, most of them being smaller in size, Approximately 30% of the cells of newborns were observed to approximate or exceed the mean in size. The distribution of megakaryocyte sizes in infancy showed almost the same tendency as that in adults. The above findings, which were obtained with addition of data, were not any different from the tendencies described in the previous report.

4) Blood dyscrasias

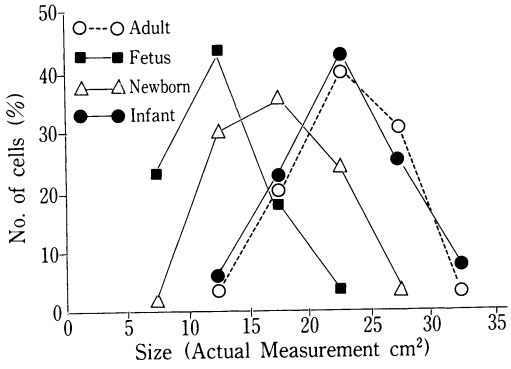

Fig. 2. Distribution of megakaryocyte size of fetal, neonatal, infantile and adult bone marrow

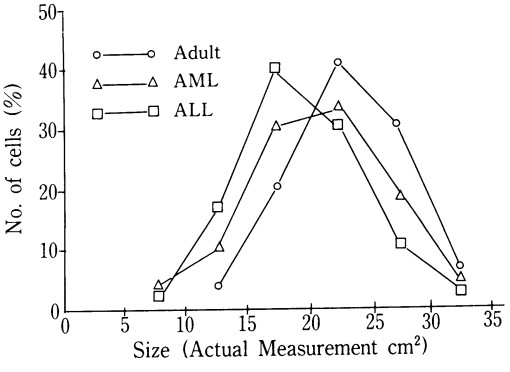

Fig. 3. Distribution of megakaryocyte size of AML, ALL, and normal adult bone marrow

Table 3. Blood Dyscrasias

Disease	Number of patient	Number of megakaryocytes	Cell size (Actual measurement cm^{2*})	(True size) μm^2
AML	11	146	19.8 ± 6.6	316.8
ALL	14	251	19.9 ± 5.6	318.4
ITP	3	58	24.6 ± 8.2	393.6

The examination results relative to the acute leukemias, AML, ALL and ITP are shown in Table 3 and Fig. 3. As evident from Table 3, the megakaryocyte sizes in both AML and ALL were significantly smaller compared with the control mean megakaryocyte size in adults. In Fig. 3, a figure showing distributions of megakaryocyte size in the acute leukemias, AML and ALL, the scope of distribution is observed to be wide in both AML and ALL and small size megakaryocytes were observed to be more numerous than the mean number of such cells in the controls.

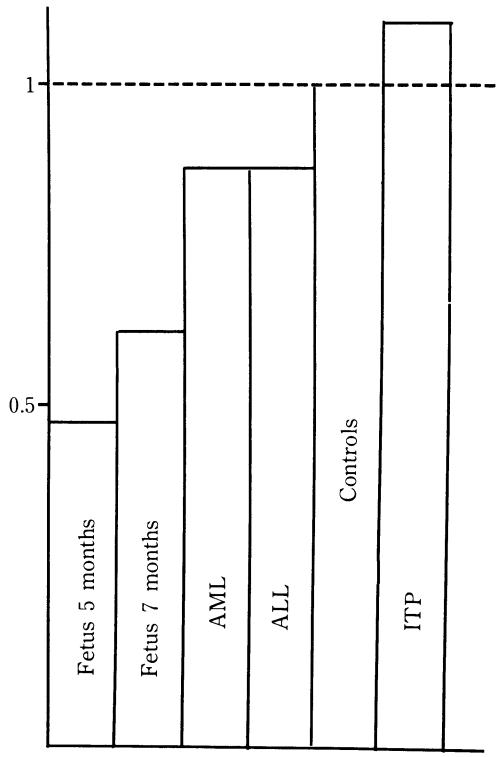

Fig. 4. Comparison of mean megakaryocyte size, in fetuses normal adults and blood diseases (normal adult as controls)

5) Comparison of mean megakaryocyte size between fetal hematopoietic stage, normal adults, and blood dyscrasias

Fig. 4, which compares mean megakaryocyte size with the mean size in normal adults as controls, shows the mean size in AML and ALL to be smaller and the mean size in ITP to be larger.

DISCUSSION

There are reports which observe the morphology of bone marrow megakaryocytes in terms of size and, in a comparison with such cells in normal individuals, describe the appearance of micromegakaryocytes in various blood dyscrasias[4,5,10,18–20]. There are also reports of appearance of micromegakaryocytes in hepatic hematopoiesis in human fetuses besides in blood dyscrasias[7,8]. Various methologies can be employed in the measurement of these sizes. As methodology for preparing (May-Giemsa staining) as employed by M. Wiesneth et al[20], fixation and paraffin imbedding of small sections of bone marrow as employed by Branehob et al[3], and use of autopsy materials as made by Enzan et al[8], and as methodology of size measurement, there is measurement after photographing as employed by M. Wiesneth et al[20], direct measurement of diameter using a micrometer as employed by Enzan et al[8], etc. In the present study, the results obtained with the addition of 11 fetus, newborn, infant and adult cases and 28 cases of blood dyscrasias, namely AML, ALL, and ITP, were examined, and no significant difference between the two were observed, neither in a comparison between the liver and the bone marrow as sites of hematopoiesis, nor in a comparison of megakaryocyte sizes between fetuses of the liver and fetuses of the bone marrow of the same months of age. This shows that there is no difference in mean megakaryocyte size by site of hematopoiesis.

Next, as regards changes in mean megakaryocyte size by fetal age, the size at 7 months of fetal age was significantly larger than that at 5 months, and it tended to increase in size with lapse of time postnatally, from newborn to infant and was observed to become hardly any different from that in adults by at least one year after birth. From the foregoing results and the results obtained in the present study with addition of about 50% of data, a tendency was observed which was almost similar to that reported previously.

The mean megakaryocyte sizes in cases of blood dyscrasias, i.e., acute leukemias, AML, and ALL, were smaller than that in adults, and with respect to AML, this can be considered to be a finding common to the report of appearance

of micromegakaryocytes in the preleukemic stage[18]. Regarding the size of megakaryocytes, the cytoplasm increases in size as megakaryocytes mature[9]. Further, it is reported that cells increase in size with increase of DNA volume and that ploidy is observed to increase with increase of nuclear size[12].

Adult marrow megakaryocytes mature up to the time of cytoplasmic disintegration, that the nuclear DNA volumes at that time can be broadly divided into three ploidy levels and that granules and demarcation membranes specific to each ploidy class are present[16]. It is reported that micromegakaryocytes in the hematopoietic stage of the human fetus are deficient in demarcation membranes and have few characteristic granules[8]. Further, megakaryocytes in the hematopoietic stage of the human fetus showed mean sizes smaller than those of megakaryocytes in blood dyscrasias, AML, and ALL, that at 5 months of fetal age being almost 1/2 as large and that at 7 months, 70% as large. Megakaryocytes in the fetal hematopoietic stage can be considered to differ considerably from also the megakaryocytes appearing in acute leukemias. Concerning such blood dyscrasias, a report states, in the cell surface of these unusual megakaryocytes active bleb formation was observed in patients with myelogenous leukemia[1] on the ultrastructure.

Considering these points, we believe that fetal hematopoietic stage, adulthood, and acute leukemia bring about differences in the function and morphology of the thrombocytes produced by megakaryocytes.

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