Quantitative Observation of Megakaryocytes in the Spleen and Bone Marrow of the Mouse: Effects of Sex, Sex Hormones, Pregnancy and Lactation*

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Summary. Megakaryocytes in the spleen and bone marrow of the mouse were quantitatively examined particularly in relation to sex.

In the splenic red pulp, megakaryocytes increase equally in number in both sexes in early life until 35 days of age. At 70 and 150 days of age, however, the number of megakaryocytes is significantly greater in females than in males, and thus a significant sex difference is apparent between the sexes. Removal of the testis causes an increase in the number of splenic megakaryocytes. In males, gonadectomized or normal, estrogen induces a marked increase in the number of megakaryocytes, whereas neither testosterone nor progesterone causes significant changes.

In females, splenic megakaryocytes show a marked increase during pregnancy. After delivery, they gradually decrease in number. The decrease is more rapid in non-lactating than in lactating mice.

In the bone marrow, no significant sex difference is evident in the number of megakaryocytes per unit area. Estrogen causes a significant increase in the megakaryocyte count also in the bone marrow.

Based on the results obtained, megakaryocytopoiesis in the hemopoietic tissue was considered from a standpoint of its relation to sex.

As is well known, megakaryocytes are present with other hemopoietic cells in the mouse throughout its life, not only in the bone marrow but also in the splenic red pulp. The splenic red pulp serves mainly as an active erythropoietic and megakaryocytopoietic tissue (Andrew, 1959; Brodsky et al., 1966; Bozzini et al., 1970; Fruhman, 1970; Schalm, Jain and Carroll, 1975; Matsumura, Sasaki and Ito, 1983). The activity in erythropoiesis in the spleen is known to be influenced by gonadal hormones, especially estrogen (Fruhman, 1966; Sasaki and Ito, 1981). As for the effect of sex hormones on megakaryocytopoiesis, it has also been reported that estrogen induces an increase in the number of megakaryocytes in the spleen and bone marrow (Landshman and Bleiberg, 1979). However, no systematic examination has been made on megakaryocytopoiesis in relation to sex.

This paper deals with the effect of sex on megakaryocytes in the spleen and bone marrow of the mouse.

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MATERIALS AND METHODS

In this study, 163 dd-mice of both sexes were used. They were maintained on a commercial mouse pellet diet (NMF, Oriental Co., Tokyo) and water ad libitum under constant environmental conditions. The mice were divided into the following four groups.

1. Normal mice: Ninety mice of both sexes were killed at 0, 20, 35, 70 and 150 days of age.

2. Gonadectomized mice: Mice of both sexes were gonadectomized under pentobarbital anesthesia at the age of 25 to 30 days, and sacrificed at 70 to 75 days of age.

3. Sex hormone-injected mice: Twenty gonadectomized males were divided into the following three subgroups: the first received five injections of 0.2 mg of estradiol benzoate; the second, five injections of 2.0 mg of progesterone; and the third, five injections of 5.0 mg of testosterone propionate. In addition, five normal males also received five injections of 0.2 mg of estradiol. For each subgroup, the mice were injected subcutaneously every two or three days starting at 60 to 63 days of age, and then killed 12 or 14 days after the first injection.

4. Pregnant and lactating mice: Female mice, 60 to 70 days old, were caged with males overnight, and the the day on which a vaginal plug was found was designated as the first day of pregnancy. The mice were killed at 5, 10 and 15 days of pregnancy, and 0, 7 and 20 days after parturition. In addition, non-lactating mothers, whose offspring had been removed immediately following birth, were killed 7 days after delivery.

The mice were killed with excess chloroform, and the spleen was excised, weighed and fixed in Zenker-formol-acetic acid solution (20:2:1) for 6 hrs. The tissue was embedded in paraffin and sectioned serially at 6 μm. The sections were stained with periodic acid-Schiff (PAS)-hematoxylin-fast green or hematoxylin-eosin. The femur was fixed and decalcified in 5% formalin-5% formic acid mixture for 1 day, then embedded in paraffin and sectioned serially at 6 μm. The sections were stained with hematoxylin-eosin.

Quantitative analysis

1. Volume of the red and white pulp of the spleen: Volumetric measurement was performed by means of a point-counting method. With the aid of a microprojector, the light microscope images of the sections were projected onto a paper with a regular lattice of points. The points on the red and white pulp of the sections projected were then counted. The point-counting was carried out at intervals of 100 μm in serial sections, and then the volume of the red and white pulp was obtained from both the number of points counted and the volume which one point represented.

2. Number of megakaryocytes: The number of megakaryocytes per unit area was obtained for both the splenic red pulp and bone marrow. Megakaryocytes were counted in sections at intervals of 50 μm. In the red pulp, the number of megakaryocytes per unit volume was calculated from both the number per unit area and the mean cell diameter measured by an optical micrometer (Williams, 1977). The total number of megakaryocytes in the spleen was estimated from the number per unit volume and the volume of the red pulp.

The statistical significance of the results obtained was evaluated by Student's t-test.
RESULTS

1. Megakaryocytes in the spleen

As shown in Figure 1, the red pulp increased markedly in volume until 35 days of age. After 35 days, the red pulp remained almost constant in volume until 150 days of age. In the spleen, megakaryocytes were generally scattered throughout the red pulp, but they appeared to vary more or less in frequency in different ages (Fig. 2).

Figure 3 presents the number of megakaryocytes per unit area in the red pulp in the two sexes at various ages. Megakaryocytes showed a marked increase in number at 35 days, when they have almost doubled in number from 20 days of age. Thereafter, megakaryocytes undergo a decrease in number. This decrease is more striking in males than in females. Thus, a significant sex difference is apparent in the number of megakaryocytes per unit area at the age of 70 (\(p < 0.001\)) and 150 days (\(p < 0.025\)). The total number of megakaryocytes in the red pulp at various ages is presented in Figure 4. The total of megakaryocytes in the red pulp increases markedly in early life, reaching a maximum at 35 days of age. Thereafter, they undergo a remarkable decline in number in males, but remain almost unchanged in females. Accordingly, in adults older than 70 days in age, megakaryocytes in the spleen are significantly greater in number in females than in males.

Effects of gonadectomy: The number of megakaryocytes per unit area in gonadectomized mice is presented in Table 1.

![Fig. 1. Volume of the splenic red pulp at various ages in postnatal life. Each point represents the mean ± SD.](image.png)

![Fig. 2. Megakaryocytes (arrows) in the splenic red pulp at 0 (a), 35 (b) and 150 (c) days of age. Hematoxylin-eosin. ×255](image.png)
In males, it is significantly larger in the gonadectomized than in the normal. Megakaryocytes are almost similar in number in the gonadectomized male to those in the normal female. In females, on the other hand, megakaryocytes are not significantly different in number between the normal and ovariectomized. In any case, no significant difference is seen in the number of megakaryocytes between the gonadectomized male and female.

**Effects of sex hormones:** The number of megakaryocytes in estrogen-, progesterone- and testosterone-injected mice is presented in Table 2. Megakaryocytes were 1.5 times as many in the estrogen-injected as in the control. In the progesterone- or testosterone-treated, on the other hand, megakaryocytes are not significantly different in number from the control.

**Effects of pregnancy and lactation:** Figure 5 shows the total number of megakaryocytes in the red pulp during pregnancy and lactation. During pregnancy megakaryocytes significantly increase in number, reaching a maximum at 15 days of pregnancy. After delivery, megakaryocytes gradually decrease in number until they have almost returned to the normal level after 20 days. In non-lactating mothers, however, mega-
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Megakaryocytes were almost the same in number at 7 days as at 20 days after delivery in lactating mothers. Therefore, the decrease of megakaryocytes is more rapid in non-lactating than in lactating mothers.

2. Megakaryocytes in the bone marrow

The number of marrow megakaryocytes per unit area at various ages is shown in Figure 6. Megakaryocytes in the marrow also exhibit a striking increase in number at 35 days of age in both sexes. They then are significantly decreased at 70 and 150 days. In the marrow, however, no significant sex difference is seen in the number of megakaryocytes at any age.

Effects of gonadectomy and sex hormones: As shown in Table 3, the number of megakaryocytes per unit area is almost the same in the normal as in the gonadectomized male. Megakaryocytes were significantly increased in number in the estrogen-injected (Table 3, Fig. 7). In the testosterone-injected specimens, however, megakaryocytes did not significantly vary in number from those in the normal and gonadectomized controls.

DISCUSSION

Effect of sex or sex hormones on megakaryocytes

The results show that megakaryocytes can be seen in the splenic red pulp throughout a mouse's life. They show a marked increase in number, reaching a maximum at 35 days of age. Then they undergo a significant decrease in number in males, whereas they remain almost unchanged in females. At 70 and 150 days of age, therefore, they show a significant difference in number between the two sexes. This finding suggests that the sex difference is associated with maturation of the gonad, testis and/or ovary.

Effects of the gonad and sex hormones on the hematopoietic organ has been studied especially in relation to erythropoesis. Erythropoesis is generally known to be stimulated not only by androgen (Fried and Gurney, 1968) but also by estrogen (Fruman, 1966; Sasaki and Ito, 1981). In mice, megakaryocytopoiesis has also been reported to be enhanced by estrogen (Landshman and Bleiberg, 1979). In male mice, as indicated in the present study, gonadectomy is followed by a significant increase in the number

| Table 1. The number of megakaryocytes per unit area in normal and gonadectomized mice |
|----------------------------------------|-----------------|-----------------|
| Number per unit area (mm²)            | Male            | Female          |
| Normal                                | 12.7±1.5        | 19.1±1.7        |
| Gonadectomized                       | 19.2±4.8        | 16.9±2.5        |

The values indicate the mean ±SD.

| Table 2. The number of megakaryocytes per unit area in sex hormone-injected mice |
|----------------------------------------|-----------------|-----------------|
| Number per unit area (mm²)            | GONADECTOMIZED  | N. GONADECTOMIZED |
|                                        | CONTROL         | INJECTED        |
|                                        | 19.2±4.8        | 34.2±5.0        |

The values indicate the mean ±SD.

| Table 3. The number of megakaryocytes per unit area in the bone marrow |
|----------------------------------------|-----------------|-----------------|
| Number per unit area (mm²)            | Normal          | Gonadectomized  |
|                                        | Male            | Male            |
|                                        | 8.9±0.8         | 9.0±0.4         |
|                                        | Testosterone    | 8.3±0.7         |
|                                        | Estrogen        | 16.5±2.2        |

The values indicate the mean ±SD.
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of splenic megakaryocytes. Thus the testis seems to exert a depressive effect on splenic megakaryocytes. In males, normal or gonadectomized, however, testosterone causes no significant change in the number of splenic megakaryocytes. On the other hand, estrogen induces a marked increase in the number of megakaryocytes in the red pulp. In progesterone-injected mice, no significant change is apparent. These results indicate that megakaryocytes in the spleen are affected by the ovarian hormones, particularly estrogen, although the influence of the testis on the spleen may not be entirely neglected.

In the bone marrow also, megakaryocytes undergo a marked increase in number until 35 days of age, then a gradual decrease equal in both sexes. Thus no significant sex difference is apparent for marrow megakaryocytes. Gonadectomy causes no change in marrow megakaryocytes either. Thus the effect of the gonad on marrow megakaryocytes is not so marked as on splenic ones. But estrogen causes a significant increase in marrow megakaryocytes, as is the case in splenic megakaryocytes.

The populations of hemopoietic cells are different between the spleen and bone marrow. In the splenic red pulp, as is well known, a majority of hemopoietic cells consists of erythroids, whereas in the bone marrow they are mainly composed of both granuloids and erythroids (SASAKI and ITO, 1978, 1981; SASAKI, MATSUMURA and ITO, 1981; MATSUMURA, SASAKI and ITO, 1983). Megakaryocytes generally coexist with erythroids in the hemopoietic tissue. For the bone marrow, the sections used for calculation of megakaryocytes contain both erythropoietic and granulopoietic foci. Thus it is probable that numerical changes in megakaryocytes, if present, may be less evident in the bone marrow than in the splenic red pulp, because the erythropoietic foci constitute a much smaller portion per unit area in the former than in the latter.

Effects of pregnancy and lactation on megakaryocytes

In mice and rats, megakaryocytes in the spleen increase in number during pregnancy (DAVIS, BEER and COOK, 1961; MARIN and McFADDEN, 1968). In man, blood platelets are also known to increase in number during pregnancy (MOR et al., 1960). As shown in the results, megakaryocytes in the spleen are significantly increased in pregnant mice. Thus pregnancy seems to exert a stimulative effect on splenic megakaryocytopoiesis. It is known that in man, there is a persistent increase in the mean level of serum estrogen during pregnancy (ROY and MACKAY, 1962). The increase of megakaryocytes in pregnancy is probably due to the increased estrogen level, because, as mentioned above, estrogen causes a significant increase in the number of megakaryocytes.
Pregnancy is known to enhance hemopoietic—particularly erythropoietic—activity in mice (FRUHMAN, 1967, 1968; SASAKI and ITO, 1980; SASAKI, MATSUMURA and ITO, 1981). It has also been observed that in the mouse spleen, erythropoiesis is stimulated by estrogen (FRUHMAN, 1966; SASAKI and ITO, 1981). As shown in the results, pregnancy, like estrogen treatment, causes a prominent increase in the number of megakaryocytes. Thus, in the mouse, estrogen exerts a marked effect on megakaryocytopoiesis as well as on erythropoiesis. In other words, megakaryocytopoiesis appears to be associated closely with erythropoiesis so far as the effect of estrogen is concerned. Therefore it seems probable that in pregnancy the production of megakaryocytes is also stimulated at least partly by estrogen, although the factors affecting erythropoiesis and megakaryocytopoiesis may be more complex.

As presented in the results, megakaryocytes exhibit a gradual decrease in number after delivery. The decrease is more rapid in non-lactating than in lactating females. It is known that in rats the estradiol concentration drops significantly within 24 hrs after parturition and then becomes gradually reduced to return to a baseline value during the postpartum period (LABHSETWAR and WATSON, 1974). It is possible that, after delivery, megakaryocytes decrease in number with reduction in the serum estrogen level. In lactating animals, activity in erythropoiesis is known to also be stimulated by prolactin (JEPSON and LOWENSTEIN, 1964, 1965, 1966). As indicated in the present results, activity in the splenic megakaryocytopoiesis after delivery differs between lactating and non-lactating mice. It is possible that prolactin also exerts a stimulative effect on megakaryocytopoiesis in the spleen of lactating mice.

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