Effects of Hypoxia on Megakaryocyte Size and Number of C3H and BALB/c Mice (43358)

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Abstract. **In an effort to explain the different platelet production capabilities of both normal and hypoxic male and female C3H and BALB/c mice, megakaryocyte size and number were determined utilizing bone marrow from both normal and hypoxic mice. The results indicate that normal BALB/c female mice have increased numbers of megakaryocytes, but of smaller size compared with either BALB/c male mice or to both sexes of C3H mice. An inverse relationship between the size and number of megakaryocytes was found in both normal and hypoxic mice; therefore, to evaluate total megakaryocyte characteristics, we calculated total megakaryocyte masses (TMM). With hypoxia, megakaryocyte number decreased, whereas megakaryocyte size increased. Despite the increase in megakaryocyte size, hypoxia caused a significant decrease in TMM ($P < 0.005$) in all mice, but female C3H mice had higher TMM ($P < 0.05$) than did female BALB/c mice. These data show that hypoxia decreases TMM in mice, and that the effect is greater in C3H mice than in BALB/c mice.** [P.S.E.B.M. 1992, Vol 199]

Several studies (1–8) have shown that hypoxia increases erythropoiesis and decreases thrombocytopoiesis in laboratory animals. The decrease in platelet counts is thought to be the result of decreased platelet production rather than shortened platelet survival, since hypoxic rats have normal platelet survival values (4), while megakaryocytes are reduced in number (4, 6, 7), as are colony forming units, megakaryocytes (9), and small acetylcholinesterase-positive cells (3) in rodents. The decreased platelet production is believed to be caused by stem cell competition between the erythrocytic and megakaryocytic cell lines (1, 3, 6, 8). A recent comparison (10) of the changes in platelet counts and platelet production rates in BALB/c mice, a mouse strain shown to have significantly lower hemoglobin O_2 affinity and lower erythrocyte 2,3-diphosphoglycerate values than C3H mice (10), and C3H mice after hypoxia showed that C3H mice consistently had greater RBC production and lower platelet production than did BALB/c mice when given the same degree of hypoxia. The degree of this inverse relationship between red blood cell and platelet production of hypoxic mice was dependent to a great extent upon the sex and strain of mice that were used, with males showing more marked changes than females (10). However, since megakaryocyte number and size were not examined, the changes in megakaryocytopoiesis responsible for the more severe thrombocytopenia in the C3H strain, and in males compared with females of both strains, could not be deduced. Here, we have compared megakaryocytes in normal males and females of both C3H and BALB/c mice and in both sexes of both strains exposed to chronic hypoxia to define the differences in megakaryocytopoiesis responsible for the different responses.

Materials and Methods

For these studies, we used bone marrows from both male and female C3H/HENHSD (C3H) and BALB/CANNHSD (BALB/c) mice that were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Peripheral blood values were reported previously (10). At the beginning of the hypoxia experiments, the mice were 4–5 weeks old and weighed approximately 15 g. The mice were enclosed in cages covered with dimethylsilicone rubber membranes, as described previously (11). After equilibration, the oxygen levels inside the cages were between 5.5% and 6.0%. To ensure that both strains of mice were exposed to the same O_2 levels, female mice from both strains were enclosed in the same cage. Likewise, both strains of male mice were

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Received June 25, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted September 24, 1991.

0037-9727/92/1993-0287$3.00/0

kept in the same hypoxic atmospheres. Mice were removed from the hypoxia chambers at 14 days and blood was collected from the retro-orbital sinus for platelet counts and packed cell volume (PCV) determinations.

The platelet counts of mice were determined manually using direct phase microscopy. PCV were obtained using a microtechnique and were read on a standard hematocrit reader.

For determination of megakaryocyte size and number, a femur from each mouse was removed and placed in a container containing 10% phosphate-buffered formalin. After fixation and decalcification, the bones were embedded in glycolmethacrylate and multiple sections of 2-μm thickness were cut from throughout the femur. After cutting, the sections were stained with hematoxylin and eosin. From 119 to 250 megakaryocyte profiles per mouse were identified and analyzed using a Digitizing Image Analysis System (Analytical Systems, Atlanta, GA) for measurement of megakaryocyte size. The methods of Weibel (12) and Cullen and McDonald (13) were used to correct for optically lost profiles. Other corrections were made for tissue shrinkage of 5%, as described previously (13). Megakaryocyte number was obtained by examining the stained sections by light microscopy at ×400. The number of megakaryocytes per high powered field was counted utilizing a minimum of 10 marrow fields/femur. The average number of megakaryocytes per high powered field was then corrected for optically lost caps and converted to megakaryocytes per unit volume (number/mm^3), as described by Cullen and McDonald (13).

Since both untreated and hypoxic mice appeared to have megakaryocyte sizes and numbers that were inversely related, total megakaryocyte mass (TMM) was calculated. For this calculation, megakaryocyte size (average diameter in μm) and number (average megakaryocytes/mm^3) were used:

$$\text{TMM } (\mu\text{m}^3/\text{mm}^3) = \frac{4}{3} \times \pi \times \left(\frac{\text{megakaryocyte diameter } (\mu\text{m})}{2}\right)^3 \times (\text{megakaryocyte/mm}^3) \quad \text{[Eq. 1]}$$

Student's t test was used to determine statistical differences between the means of these data.

Results

Table I shows the effects of hypoxia on platelet counts and packed cell volumes of both male and female C3H and BALB/c mice. Although previously reported (10), these data are presented here for comparison purposes. As shown, the platelet counts of untreated, female BALB/c mice were significantly lower than the values for the other mice presented ($P < 0.05$). Although hypoxia caused significant decreases in platelet counts of all mice ($P < 0.0005$), hypoxic male and female C3H mice had lower platelet counts than did hypoxic BALB/c male and female mice ($P < 0.0005$). Table I also records the PCV of these same mice. Untreated male C3H mice had lower PCV than did female C3H mice ($P < 0.05$), but PCV of untreated BALB/c male mice did not differ significantly from untreated BALB/c female mice. In addition, the untreated C3H male and female mice had lower PCV than did BALB/c mice ($P < 0.05$–$P < 0.0005$). Hypoxia caused significant increases in PCV of both sexes and strains of mice ($P < 0.0005$), with greater responses occurring in C3H mice than in BALB/c mice ($P < 0.05$–$P < 0.005$).

Figure 1 records the results of measuring megakaryocyte size and number in bone marrow of the same mice presented in Table I. Compared with untreated mice, megakaryocyte size was significantly elevated ($P < 0.05$) in hypoxic C3H male and BALB/c female mice, but not in hypoxic C3H female or BALB/c male mice (Fig. 1A). However, megakaryocyte number (Fig. 1B) was significantly ($P < 0.005$–$P < 0.0005$) reduced in all mice after 14 days of hypoxia. An inverse relationship between megakaryocyte size and number was found; this pattern was more pronounced in C3H mice than in BALB/c mice. In addition to changes in megakaryocytes that were caused by hypoxia, several significant differences were also found when comparing different sexes of the mice. Although megakaryocyte size did not differ in C3H male and female mice, megakaryocytes were smaller ($P < 0.005$) in untreated BALB/c female mice than in untreated BALB/c male mice. Likewise, megakaryocyte number was higher ($P < 0.005$) in female BALB/c mice compared with their male counterparts. The effects of testing for mouse strain differences showed that megakaryocyte sizes of untreated male C3H and BALB/c mice were significantly different ($P < 0.01$), as were significant differences ($P < 0.005$) found between untreated female C3H and BALB/c mice. Hypoxic male and female C3H mice had larger megakaryocytes ($P < 0.005$) than did male and female BALB/c mice. In addition to changes in megakaryocyte size, hypoxia also caused significant alterations in the megakaryocyte numbers of these mouse strains, i.e., C3H male and female mice had lower megakaryocyte numbers than did BALB/c mice ($P < 0.05$–$P < 0.0005$). In untreated mice, megakaryocyte numbers were the same for male C3H and BALB/c mice, but BALB/c female mice had greater numbers of megakaryocytes ($P < 0.005$) than did C3H female mice.

Since Figure 1 illustrated that both before and after hypoxia, megakaryocyte sizes and numbers were inversely related, total megakaryocyte masses were calculated (Table II). The data illustrate that although hypoxia caused significant increases in megakaryocyte sizes, the TMM were significantly reduced by hypoxia ($P < 0.005$) in all mice. The only significant difference among strains and sexes was that female C3H mice had

Table I. Effects of Hypoxia on Platelet Counts and Packed Cell Volumes of Male and Female C3H and BALB/c Mice[a]

Sex and strain of mice	Platelet counts ($mm^3 \times 10^{-5}$) Days of hypoxia		PCV (%) Days of hypoxia	
	0	14	0	14
Male C3H	9.5 ± 0.2	0.9 ± 0.3[b]	44.7 ± 0.7	76.6 ± 0.2[b]
Female C3H	9.6 ± 0.3	1.2 ± 0.2[b]	47.6 ± 0.4[c]	78.9 ± 0.5[b,c]
Male BALB/c	9.2 ± 0.4	4.1 ± 0.3[b,d]	48.8 ± 0.4[d]	69.9 ± 0.3[b,d]
Female BALB/c	7.8 ± 0.4[c,e]	4.4 ± 0.3[b,d]	49.7 ± 0.4[e]	67.0 ± 0.4[b,d,f]

[a] These data were taken from Ref. 10; they are presented as mean ± SE, and each point is the average of five to six mice.
[b] Values for control mice were significantly different from mice enclosed in hypoxic cages: $P < 0.0005$.
[c] Within strains of mice, values for female mice were significantly different from those for male mice: $P < 0.05$.
[d] Within sexes of mice, values for BALB/c mice were significantly different from values for C3H mice: $P < 0.0005$.
[e] Within sexes of mice, values for BALB/c mice were significantly different from values for C3H mice: $P < 0.05$.
[f] Within strains of mice, values for female mice were significantly different from those for male mice: $P < 0.0005$.

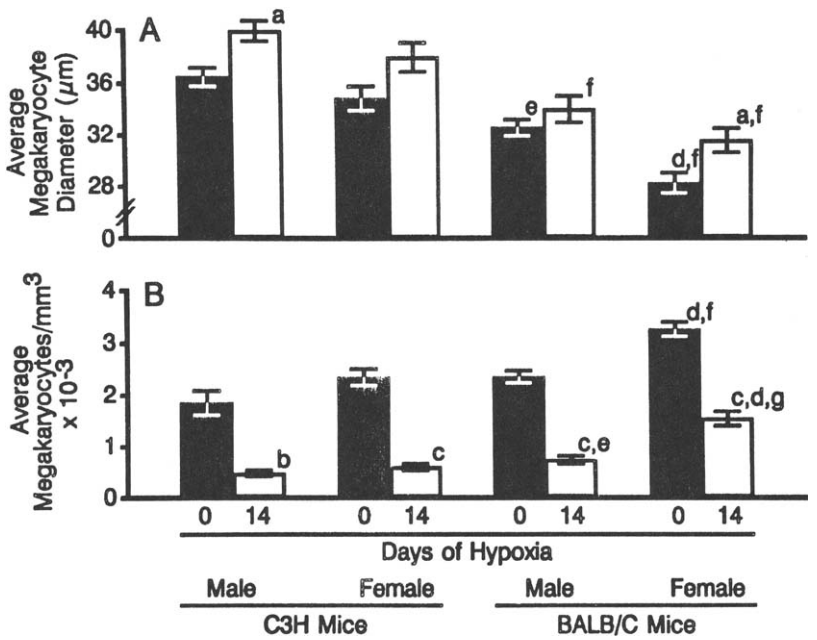

Figure 1. Effects of hypoxia on megakaryocyte (A) size and (B) number of male and female C3H and BALB/c mice. Solid bars represent the values obtained from mice kept at ambient O_2 levels, and the open bars record the results of hypoxic mice. Mice were enclosed in cages covered with silicone rubber membranes for 14 days (5.5–6.0% O_2), and megakaryocyte size and number of femoral bone marrow were determined by the methods of Cullen and McDonald (13). Vertical bars represent SE; a total of 119–250 megakaryocytes from each of four mice were measured at each data point. Within sexes and strains of mice, values for mice kept at ambient air (21% O_2, Day 0) were significantly different from those for mice enclosed in hypoxic chambers: [a] $P < 0.05$; [b] $P < 0.005$; and [c] $P < 0.0005$. Values for male mice were significantly different from those for female mice: [d] $P < 0.005$. Values for C3H mice were significantly different from those for values of BALB/c mice: [e] $P < 0.05$; [f] $P < 0.005$; and [g] $P < 0.0005$.

higher TMM than did female BALB/c mice ($P < 0.05$) before hypoxia and lower TMM ($P < 0.05$) after 14 days of hypoxia.

Discussion

Our previous studies (10, 14) showed that hypoxia caused greater changes in male mice than in female mice, with male C3H mice showing the greatest increase in PCV and the lowest platelet counts of all mice tested. As reported (10), platelet counts were lower in untreated female BALB/c mice than in their male counterparts, but the PCV were not significantly different (Table I). Moreover, the number of megakaryocytes in the bone marrow of all mice was reduced by hypoxia (Fig. 1). C3H male and BALB/c female mice had significantly larger megakaryocytes after hypoxia when compared with mice kept at ambient air. Hypoxia did not cause significant alteration in the megakaryocyte sizes of C3H female and BALB/c male mice. These data illustrate that in both normal mice and mice exposed to hypoxia, an inverse relationship existed between megakaryocyte size and number. Calculation of total megakaryocyte masses showed that, except for female C3H mice, the TMM was the same for the other groups of mice, both normally and after hypoxia (Table II). Therefore, these data show for the first time that the absolute total

Table II. Effects of Hypoxia on Total Megakaryocyte Mass of Male and Female C3H and BALB/c Mice[a]

Sex and strain of mice	Total megakaryocyte mass ($\mu m^3/mm^3 \times 10^{-6}$) Days of hypoxia	
	0	14
Male C3H	46.13 ± 6.06	15.18 ± 1.42[b]
Female C3H	51.12 ± 1.72	16.87 ± 2.58[b]
Male BALB/c	41.53 ± 1.39	21.19 ± 3.34[b]
Female BALB/c	38.36 ± 2.89[c]	24.52 ± 1.16[b,c]

[a] Data are expressed as mean ± SE; each point is the average of four mice. For the method of calculation of total megakaryocyte mass see Materials and Methods.
[b] Values for hypoxic mice were significantly lower than values for normal mice: $P < 0.005$.
[c] Values for BALB/c female mice were significantly different from those for C3H female mice: $P < 0.05$.

megakaryocyte mass is reduced in mice by hypoxia. Although female BALB/c mice had significantly smaller megakaryocytes with greater numbers than the other mice, the TMM of different strains and sexes of mice were similar.

We also found previously (10) poorer responses to hypoxia in BALB/c mice than in C3H mice, presumably because BALB/c mice were shown in this earlier work to have higher P50s (right-shifted O_2 dissociation curves) and lower erythrocyte 2,3-diphosphoglycerate values than C3H mice, indicating a lower hemoglobin O_2 affinity for BALB/c mice. The right-shifted hemoglobin dissociation curve of BALB/c mice facilitates oxygen unloading without compromising O_2 uptake from the environment. The decreased hemoglobin affinity for oxygen, which probably led to higher O_2 levels in tissues of BALB/c mice than in C3H mice, resulted in smaller increases in PCV, because of the reduced need for erythropoietin production, and only moderate degrees of thrombocytopenia (10). Thus, in agreement with the stem cell competition hypothesis, we found that the platelet counts of BALB/c mice were not as severely reduced by hypoxia as was found in C3H mice.

The present data also show an inverse relationship between megakaryocyte size and number, i.e., C3H male mice normally have the largest megakaryocytes, but the lowest concentration of these cells compared with BALB/c female mice. BALB/c mice normally have the smallest sizes with the largest numbers of megakaryocytes in their bone marrow when compared with other mice (Fig. 1). Mice appear to partially compensate both normally and after hypoxia for reduced numbers of megakaryocytes in their marrows by increasing their megakaryocyte sizes. However, it should also be emphasized that mice exposed to hypoxia had significantly smaller TMM than did normal mice. Other previous work showed that laboratory animals exposed to hypoxia have reduced megakaryocyte concentrations (4, 6, 7), a decreased number of colony forming units-megakaryocyte (9), and a reduction in the number of small acetylcholinesterase-positive cells (3). We now report for the first time that hypoxia causes an absolute decrease in megakaryocyte mass, a finding that supports the stem cell competition hypothesis between the erythrocytic and megakaryocytic cell lines.

The present work shows that hypoxia reduces the number of megakaryocytes in the marrow of mice and increases their mean size, which is presumably an effort to compensate for the megakaryocytopenia and/or thrombocytopenia. However, hypoxia reduced TMM, indicating an absolute decrease in megakaryocytes in the marrow of mice exposed to hypoxia.

This work was supported by Grants HL14637 and HL31598 from the National Heart, Lung, and Blood Institute; Grants CA21765 and CA20180 from the National Cancer Institute, National Institutes of Health, Public Health Service, Department of Health and Human Services; and by the American Lebanese Syrian Associated Charities.

The authors gratefully acknowledge the technical support of Carol Swearingen and the stenographic aid of Wanda Aycock.

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