Inherited Thrombocytopenia Caused by Reduced Platelet Production in Mice With the Gunmetal Pigment Gene Mutation

By Edward K. Novak, Madonna Reddington, Li Jie Zhen, Paula E. Stenberg, Carl W. Jackson, Michael P. McGarry, and Richard T. Swank

Hereditary macrothrombocytopenia and prolonged bleeding times are associated with the recessive mouse pigment dilution gene gunmetal (gml). Other platelet abnormalities include a mild storage pool deficiency and abnormal expression of two low-molecular-weight guanosine triphosphate binding proteins. These studies were designed to further elucidate the cause of the macrothrombocytopenia. The life span of gml dilution in the mouse is inherited as a recessive trait that maps to chromosome 14. Analy- ses of genetic backcrosses have detected no gross chromosomal abnormalities in mutant mice in the region of chromosome 14 surrounding the gunmetal gene (R.T.S., unpublished data, August 1994). The mutation, therefore, likely represents minimal changes in gene structure (eg, base pair substitutions, small deletions, or insertions). The gml mutation causes macrothrombocytopenia and a prolonged bleeding time. Proteins of platelet alpha granules such as platelet factor 4, von Willebrand factor, and fibrinogen are deficient. Also, gml mice have abnormal expression of two platelet low-molecular-weight guanosine triphosphate (GTP)-binding proteins. Recent research indicates that platelet low-molecular-weight GTP-binding proteins impart specificity to the membrane budding and fusion reactions involved in intracellular vesicle sorting. This is of interest because platelet formation involves extensive megakaryocyte membrane budding and fusion. No significant quantitative or qualitative abnormalities in peripheral blood cells, other than platelets, have been detected in mutant mice. The primary gene defect responsible for the gml mutation remains unknown.

The gml mutation is similar in certain respects to the human gray platelet syndrome in that both exhibit reduced platelet numbers, large platelet volumes, deficiencies of platelet alpha-granule proteins, and appearance of alpha-granule proteins in plasma. Likewise, there are striking similarities between the inherited gunmetal abnormalities and those recently described in the Wistar-Furth rat. Each has macrothrombocytopenia together with partial deficiencies in alpha-granule components.

The following results indicate that the thrombocytopenia of gml mice is caused by lowered rates of platelet synthesis and suggest that both qualitative and quantitative abnormalities of megakaryocytes are responsible for the abnormal rates of platelet production.

MATERIALS AND METHODS

Animals. Gunmetal (gml/gm) and control (gml/+) mice on the C57BL/6J inbred strain background were obtained from The Jackson Laboratory (Bar Harbor, ME) and subsequently maintained and bred in the mouse colony at Roswell Park Cancer Institute (Buffalo, NY). These are coisogenic mice, as the mutation originally arose on the C57BL/6J inbred strain. Mutant and control (gm/+mice were maintained by breeding heterozygous gm/+mice by homozygous gm/gm mice.

Platelet production and survival. Rates of synthesis of platelets were measured by injection of 30 μCi of 35S into each of six normal gm/+, wild-type gm/+, and six gml/gm mice. At 24 hours, mice were killed by anoxia with CO2 and blood was immediately collected into a syringe via cardiac puncture with Na2EDTA at a final concentration of 12.5 mmol/L. To ensure that the large platelets of gml/gm mice were collected, all platelets were purified by Percoll density gradient centrifugation as described by Jackson et al. Platelet fractions from the Percoll gradient were diluted sixfold with phosphate-buffered saline (PBS) and pelleted at 1,000g for 10 minutes. Contaminating red blood cells were removed by suspension in 1 mL of 1% amm-
nium oxalate followed by centrifugation at 1,000g for 10 minutes. The resulting platelet pellets were resuspended in 250 μL PBS and sonicated, and aliquots were analyzed by scintillation counting. Initial and final platelet counts were determined by phase contrast microscopy, and percent 35S incorporation was calculated as described by McDonald.17

To compare platelet survival in normal and mutant mice, four normal gm+/ and four gm/gm mice were irradiated with 9 Gy 60Co. Blood (10 μL) was collected daily from an incision at the tip of the tail into a pipette that had been rinsed with 3.8% sodium citrate. The blood was immediately diluted into 1% ammonium oxalate to lyse erythrocytes, and platelets were counted in a hemocytometer by phase contrast microscopy. Samples were determined to be free of any platelet aggregates by phase contrast microscopy. Blood was collected daily for 8 consecutive days. The mice appeared healthy throughout this period, after which they were killed by CO2 anoxia.

Blood was collected via cardiac puncture into acid citrate dextrose containing 8 pmol/L PgE1. The blood from three platelet analysis, blood was collected into acid citrate dextrose containing 8 pmol/L PgE1. Blood was collected daily for 8 consecutive days. The mice appeared healthy throughout this period, after which they were killed by CO2 anoxia.

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To test for successful engraftments, a rapid method with oligonucleotide primers specific for the X and Y chromosomes, respectively, in the polymerase chain reaction (PCR) assay was used. After final hematologic analyses of engrafted mice, they were killed, and spleen cells, predominantly lymphocytes, were isolated by passage of spleens through a 25-μm wire mesh. Cells were washed with PBS, and DNA was isolated from them by the method of Mann et al.26 Primers on the X chromosome (Supplied by Dr Verne Chapman, Roswell Park Cancer Institute) are specific for the 3' region of HPRT. The forward primer was 5' TAGGAAAGAGCATGTGCTGG 3' and the reverse primer was 5' CTGAAAATGTCGAAAATACACC 3'. The Y chromosome primers were 5' AAGATACAACTATGATTTACATGG 3' and 5' CCTATGAAATCCCTTTGCTGC 3' and were devised by Beth Simpson at the Whitehead Institute, Massachusetts Institute Technology (Boston, MA).

The PCR reaction mix (50 μL) included 50 ng DNA, 0.16 mmol/L deoxynucleoside triphosphates (dNTPs), 20 μmol/L of each primer, 0.4 U Taq polymerase (Boehringer Mannheim, Indianapolis, IN) in 10 mmol/L Tris, 1.5 mmol/L MgCl2, and 50 mmol/L KCl, pH 8.3. PCR reaction conditions were 5 minutes at 95°C, followed by 30 cycles of 1 minute at 94°C, 1 minute at 58°C, and 2 minutes at 72°C. A 6-minute elongation step at 72°C was used after the last cycle.

Reaction products were electrophoresed on 1% agarose gels that were stained with ethidium bromide and photographed with Polaroid type 55 film (Cambridge, MA). The Y chromosome-specific product is 600 bp, and the X chromosome-specific product is 300 bp. Negatives were scanned and quantitated in a Helena Quik Scan Jr densitometer (Beaumont, TX). A standard using mixed proportions of male and female spleen cells was analyzed to establish the limits of detection for mixed chimerism.

For megakaryocyte enumeration and sizing. Bone marrow suspensions were prepared in PBS, and cells were centrifuged onto slides with a Shandon Cytospin 2 centrifuge (Sewickley, PA). Cells were expressed from spleen and cytocentrifuged as above. Cells were fixed and stained with Leukostate (Fisher Diagnostics, Orangeburg, NY) before enumerating total megakaryocytes. In some cases, sections of spleen were fixed in 10% buffered formalin and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin and counted. Cells greater than 18 μm in diameter and containing a polyploid nucleus were included in the analyses.

Megakaryocyte diameters were determined with an ocular under high power. Two measurements were made at right angles, with one measurement including the longest diameter of the cell. The mean cell diameter was calculated.

Megakaryocyte ploidy analysis. The DNA distribution of megakaryocytes in unfractonated marrow was determined as previously described.21 Marrow from both femurs of each mouse was washed out with 1 mL of CATCH medium.22 A monoclonal antibody (4A5) with high specificity for mouse platelets and megakaryocytes (provided by Dr Samuel Burstein, University of Oklahoma, Norman, OK) was used as the primary antibody for labeling megakaryocytes for the flow cytometric analysis. Fluorescein isothiocyanate (FITC)-goat-antimouse IgG (Fab')2 (TAGO, Burlingame, CA) was used as the secondary antibody. DNA was stained with propidium iodide in hypotonic citrate.23 Using an Epics 753 flow cytometer (Coulter Electronics, Hialeah, FL) and two-color flow cytometry,24 DNA content of all 4A5-positive cells was measured (DNA content from 2N to 64N). However, for the purposes of this study, only those cells with DNA contents ≥8N were considered, as the proportions of 2N and 4N platelet-antibody-positive cells detected in unfractionated marrow is very dependent on the selection of the green fluorescence gate. The proportion of cells in each ploidy class was determined by integrating the number of cells under each DNA peak.

RESULTS

Reciprocal bone marrow transplantation. To determine if the mutant hematologic effects were due to abnormalities in platelet marrow progenitor cells, reciprocal bone marrow transplantation was conducted between mutant gm/ and normal gm/+ mice (Table 1). This engraftment is facilitated by the congenic nature of the gunmetal mutation, which guarantees compatibility in transplantation antigens. When gm/ marrow was transplanted into normal gm/+ recipients, recipient mice uniformly had prolonged bleeding times, decreased platelet counts, and increased platelet volumes. Conversely, transplantation of gm/+ marrow into mutant gm/
Thrombocytopenia in Gunmetal Mice

Table 1. Bleeding Times, Platelet Counts, and Mean Platelet Volumes in Recipient Mice After Bone Marrow Transplantation

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Bleeding Time (min)</th>
<th>Platelet Counts (×10^12/L)</th>
<th>Mean Platelet Volume (FL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm/+</td>
<td>gm/+</td>
<td>2.7 ± 0.7 (3)</td>
<td>733 ± 24 (2)</td>
<td>6.6 ± 0.5 (2)</td>
</tr>
<tr>
<td>gm/gm</td>
<td>gm/gm</td>
<td>&gt;15 (3)</td>
<td>312 ± 25 (3)</td>
<td>7.3 ± 0.20 (2)</td>
</tr>
<tr>
<td>gm/gm</td>
<td>gm/+</td>
<td>13.5 ± 0.9 (7)</td>
<td>304 ± 15 (7)</td>
<td>7.3 ± 0.14 (7)</td>
</tr>
<tr>
<td>gm/+</td>
<td>gm/gm</td>
<td>4.0 ± 0.7 (8)†</td>
<td>488 ± 36 (7)†</td>
<td>6.3 ± 0.10 (7)†</td>
</tr>
</tbody>
</table>

The significance tests compare the results of the reciprocal transplantation (lines 3 and 4). Values are the mean ± SEM of the number of mice in parentheses.

* P < 0.001.
† P = 0.01.

gm recipients normalized all three parameters in recipient mice. In control experiments, normal recipients transplanted with normal marrow and mutant recipients transplanted with mutant marrow retained their expected phenotypes.

To distinguish donor engraftment from endogenous regeneration, a procedure based on the PCR technique was used. This technique rapidly distinguishes male donor-derived DNA from female recipient-derived DNA. DNA amplification with Y chromosome- and X chromosome-specific primers, used simultaneously, results in a 600-bp Y chromosome-specific product and a 300-bp X chromosome-specific product (Fig 1A). DNA mixtures from suspensions with defined percentages of cells of male and female origin were amplified and separated on polyacrylamide gels (Fig 1A). When the ethidium bromide-stained products were scanned in a densitometer, a high correlation was observed between the expected and observed percentages of Y chromosome product. When DNA samples from spleen cells of female recipients used in the gunmetal experiment of Table 1 were analyzed by PCR, the expected concentration of Y chromosome-specific product was visible in all cases (Fig 1B), indicating successful engraftment from male donors.

This PCR-based test for successful engraftment was found to be both rapid and reliable. Its sensitivity exceeds that of morphologic and biochemical procedures used previously.

Rates of synthesis of platelets. The bone marrow transplantation experiments indicated that a possible explanation for the lowered platelet counts in gunmetal mice is an alteration in platelet production. To directly compare synthetic rates of platelets in normal (gm/+ and gm/gm) mice, the rates of incorporation of [35S]sodium sulfate into platelets were measured (Table 2). The percent [35S] incorporated into platelets of gm/gm mice was only 26% of that of gm/+ mice. This relative rate of synthesis was similar to the lowered number of platelets (29% of normal) observed in the same mutant mice. Thus, the thrombocytopenia of the mutant mice can be directly accounted for by a decrease in the rate of platelet synthesis.

The platelet counts of both normal and mutant mice are higher in the experiment reported in Table 2 as compared with that in Table 1. The probable explanation is that different techniques (manual and automated, respectively) were used. The automated counter used in the experiment reported in Table 1 was calibrated for routine clinical specimens and, therefore, undoubtedly underestimates the number of murine platelets, which are substantially smaller than human platelets.

Rates of degradation of platelets. Another possible explanation for the thrombocytopenia of gunmetal mice is lowered platelet survival. This possibility is less likely given the above determination that mutant platelet synthetic rates are decreased. Nevertheless, platelet survival in normal and mutant mice was compared after irradiation of mice with 9.0 Gy 60Co (Fig 2). Because this dose of irradiation is cytocidal to megakaryocyte progenitor cells, megakaryocyes of normal and, eventually, platelet synthesis cease in treated mice. As a consequence, the rate of loss of platelets from peripheral

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**Fig 1.** Amplification of specific regions of X and Y chromosomes by the PCR. DNA was isolated from spleen cells (predominantly lymphocytes) of male and female mice and amplified by PCR methods. (A) Male and female DNA were mixed in the ratios indicated (female: male) before amplification. (B) DNA was isolated from spleen cells (predominantly lymphocytes) of female recipients that had received bone marrow transplants from male donors of the indicated genotype. Products were electrophoresed on polyacrylamide gels and detected by staining with ethidium bromide. Standards are a mixture of markers of known molecular weight. Sizes in base pairs of selected standard bands are indicated.
Fig 2. Platelet survival in normal (gm/+). Day 0. Platelet counts were thereafter determined daily. Values represent the mean ± SD of four mice. Similar results were obtained in two independent experiments.

**Table 2. Rates of Incorporation of [35S] Sulfate in Normal (gm/+).**

<table>
<thead>
<tr>
<th>Incubation</th>
<th>% Incorpoated (×10−5)</th>
<th>Platelet Counts (×10−6/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm/+</td>
<td>9.5 ± 0.36</td>
<td>1,890 ± 110</td>
</tr>
<tr>
<td>gm/gm</td>
<td>2.5 ± 0.24*</td>
<td>557 ± 35*</td>
</tr>
</tbody>
</table>

[^35S] sulfate (30 μCi) was injected into each of six normal and six mutant mice. Platelets were purified after 24 hours, and the [%[^35S]] incorporated was calculated as described in Materials and Methods. Values represent mean ± SEM. *P = 0.01.

blood of irradiated mice is a measure of platelet survival. It was expected that megakaryocytes existing before the irradiation would continue to produce platelets for a limited time interval. The results (Fig 2) verify that there is a 1 to 2-day lag period before measurable loss of platelets from peripheral blood. For both normal and mutant mice, platelet levels subsequently decreased by a first-order process. Platelet half lives determined from the slopes of these curves are similar in normal and mutant mice (1.5 and 1.8 days, respectively).

**Morphologic abnormalities of megakaryocytes and platelets.** Collectively, the above experiments strongly suggest that the platelet producing cells of gunmetal marrow are abnormal. To test for qualitative differences in these cells, bone marrow megakaryocytes of homozygous gm/gm mutants and normal heterozygous controls were examined by electron microscopy. Control gm/+ megakaryocytes (Fig 3) appeared completely normal, with the demarcation membrane system and cytoplasmic organelles homogeneously distributed within the cytoplasm. In contrast, gm/gm megakaryocytes showed a number of abnormal features (Fig 4). First, the demarcation membranes were not homogeneously distributed. Areas of abnormal membrane complexes were apparent, while other areas exhibited an absence of the demarcation membrane system. Second, some cells showed areas of organelles (ER, Golgi) separated from areas of demarcation membranes. Third, the incidence of emperipolesis, i.e., other cells trafficking through the megakaryocyte, was greatly increased as compared with normal megakaryocytes (Fig 4). No megakaryocytes were observed in the process of lysis, when observed by either light or electron microscopy.

Platelets produced from mutant megakaryocytes also had obvious abnormalities. Mutant gm/gm platelets (Fig 5B) were more heterogeneous in size than normal gm/+ platelets (Fig 5A). Larger platelets were apparent; some platelets had unusual membrane complexes; and many mutant platelets had unusual, elongated, striated inclusion granules (Fig 5C), while others contained profiles of Golgi complex, which were not observed in normal platelets. In addition, granule number and size were reduced from normal. Immunolabeling with platelet factor 4 antibodies (data not shown) suggests the elongated inclusion bodies are modified alpha granules. The inclusion bodies resemble Weibel-Palade bodies in their elongated shape, but the internal organization of the two granules differs.

**Megakaryocyte counts and sizes in marrow and spleen.** To determine if there were a mutant effect on megakaryocyte number and/or size, megakaryocytes were examined by high-power microscopy in spleen and marrow of recipient mice (Table 3). An approximately fourfold increase in megakaryocyte number was observed in both marrow and spleen of gunmetal mice. Megakaryocyte mean diameters did not vary significantly by genotype or tissue (Table 3).

Microscopic analysis showed no visible megakaryocytes in lung or liver sections.

**Megakaryocyte ploidy analyses.** Bone marrow megakaryocyte ploidy analyses of normal and mutant mice were performed by fluorescence-activated cell sorter (FACS) procedures using the platelet-specific rat monoclonal 4A5 antibody of Dr Samuel Burstein. 22 The megakaryocyte ploidy distribution of gunmetal mice was shifted to the right, with a doubling of the percentage of 32N megakaryocytes and a concomitant decrease in 8N cells (Table 4).

**DISCUSSION**

These experiments further define the cause of the inherited macrothrombocytopenia in gunmetal mice. The [35S] incorporation experiments directly establish that the thrombocytopenia is accounted for by a corresponding decrease in the rate of synthesis of platelets. The results of platelet survival experiments reinforce this conclusion, as platelet half-lives were not significantly different in normal and mutant mice. Reciprocal bone marrow transplantation experiments provided additional evidence consistent with an impairment in
THROMBOCYTOPENIA IN GUNMETAL MICE

Fig 3. Transmission electron micrograph of a normal (gm/+)
mouse megakaryocyte. The cell is normal in size, and the demar-
cation membrane system (DMS) is homogeneously distributed
throughout the cytoplasm. Original magnification (OM) x 7,000;
N, nucleus.

Platelet production in mutant mice. The thrombocytopenia
of mutant mice was corrected by transplantation with normal
marrow, and normal mice became thrombocytopenic when
transplanted with mutant marrow. Platelet size and bleeding
times were likewise controlled by the marrow source. This
indicates that a defect in a marrow progenitor cell is responsi-
ble for all the defects of hemostasis in gunmetal mice.

Indeed, it was found that megakaryocytes of gunmetal
marrow are morphologically aberrant. Most striking was the
presence of unusual membrane complexes that replaced the
ordered demarcation membrane system of normal megakary-
ocyes. Another prominent feature was an increase in the
number of emperipolesed cells in mutant megakaryocytes.
The significance of the increased megakaryocyte emperi-
polesis is uncertain, though it is of interest that others have
noted an increased megakaryocyte emperipolesis in patients
with altered platelet production. It has been suggested that
chemotactic factors released from leaky mutant megakaryo-
cyte alpha granules recruit other cells within the mutant
cytoplasm. This is a likely interpretation of the increased
emperipolesis in gunmetal megakaryocytes, as alpha granule
components are reduced in platelets and increased in plasma
of mutant mice. The abnormal mutant megakaryocyte mor-
phology extends to daughter platelets, which are more het-
erogeneous in size and contain granules with a characteristic
elongated and striated appearance. Preliminary evidence sug-
gests these are modified alpha granules. The abnormal mega-
nyocyte membrane complexes are occasionally seen in mu-
tant daughter platelets. Their presence, therefore, likely
reflects a defect in the ordered distribution of membranes
during platelet formation. The above-described morphologic
abnormalities in mutant megakaryocytes and platelets may
be intimately related to the observation that low-molecular-
weight GTP-binding proteins are abnormally expressed in
gunmetal platelets, in that the morphology of other intracel-
lar organelles, such as the Golgi apparatus, is dramatically
altered by expression of abnormal GTP-binding proteins.

Significant quantitative abnormalities in megakaryocytes
were likewise obvious in mutant mice. Both bone marrow
and spleens of mutant mice had about four times the number
of megakaryocytes found in normal controls. Apparently, a
feedback mechanism similar to that observed in experimen-
tally induced thrombocytopenia senses the deficiency of platelets in peripheral blood of gunmetal mice and responds by increasing megakaryocyte production. Paradoxically, a fourfold increase in numbers of gunmetal megakaryocytes still yields a substantial deficiency in the rate of production of platelets, as measured by sulphate incorporation. This demonstrates that platelet production from mutant megakaryocytes is very inefficient. On a per megakaryocyte basis, platelet production in gunmetal mice is deficient, by an order of magnitude, when compared with normal megakaryocytes.

It is possible that the impaired platelet production in gunmetal is accompanied by either late or early disturbances of megakaryocyte maturation. Ploidy analyses established that there is a small right shift in megakaryocytes in mutant mice, with a twofold increase in the percentage of 32N cells and half the normal percentage of 8N cells. A similar shift to higher ploidy classes is seen in experimentally induced thrombocytopenia. It is likely that both observations result from autoregulatory mechanisms controlling platelet levels. The shift to higher ploidy classes observed in gunmetal marrow indicates that regulation of megakaryocyte endoreduplicatory mechanisms in mutant mice is normal. The cause of the lowered mutant platelet production may be related to abnormal membrane maturation and/or fragmentation in megakaryocytes. The same defect may account for the abnormalities in mutant platelet size and alpha granules.

A total of 13 other mouse mutations affecting platelet function have been described. They exhibit prolonged bleeding times and other typical symptoms of platelet storage pool deficiency (SPD). The gunmetal mutation is similar to this group of mutants in that it is an autosomal, recessively inherited mutation causing diluted coat color and has a mild platelet SPD. Its hemostatic characteristics are unique among this group, however, in that it is the only mutation with abnormalities in rates of platelet production.

In several respects, the properties of the gunmetal mouse resemble those recently described in the Wistar-Furth rat. In each, an autosomal recessive gene causes macrothrombocytopenia, an irregular megakaryocyte demarcation membrane system with abnormal membrane complexes, in-
Fig 5. Transmission electron micrographs of gm/+ and gm/gm platelets. (A) The gm/+ mouse platelets are normal in size, shape, and appearance. OM x 16,000. (B) The gm/gm mouse platelets show greater size heterogeneity, and some contain membrane complexes (arrow). OM x 16,000. (C) The gm/gm platelet, showing elongated granules with a striated pattern in the granule matrix (arrows). OM x 43,000. DB, dense body; αG, alpha granule; MT, mitochondrion.

Increased emperipolesis in megakaryocytes, and reduced platelet alpha-granule protein contents. Each phenotype is controlled by an intrinsic abnormality in marrow progenitor cells. Several facts suggest, however, that different genes may be responsible for the abnormalities in the two animal models. For example, an increase in marrow megakaryocyte numbers and a shift to higher megakaryocyte ploidy occur only in gunmetal. Elongated striated granules occur only in gunmetal platelets. Also, platelet volumes increase with age only in the Wistar-Furth rat.

The gunmetal mouse and the Wistar-Furth rat are two animal models that may prove useful in deciphering the little understood processes of the subdivision of the megakaryocyte cytoplasm into platelets and the formation of alpha granules. An absence of the typical megakaryocyte demarcation membrane system together with abnormal membrane complexes occurs in both. The mutant platelets produced by these cells are reduced in number, have increased size heterogeneity, are larger than normal, and have reductions in alpha-granule components. Molecular characterization of the responsible genes affected in each model should, therefore, show components important to all these processes.

Some features of the gunmetal phenotype resemble those of inherited human syndromes. For example, increased platelet size with reduced platelet counts occurs in Mediterranean macrothrombocytopenia, gray platelet syndrome, and other thrombocytopenias. Several of these human syn-

| Table 3. Quantitation of Megakaryocytes in Mutant (gm/gm) and Normal (gm/+ ) Mice |
|-------------------------------|-------------------|
|                               | gm/gm             | gm/+              |
| No. of megakaryocytes/10⁶ cells |                    |                    |
| Femoral marrow                | 57 ± 13*          | 14 ± 1.5          |
| Spleen                        | 14 ± 2.4*         | 3.8 ± 1.1         |
| Mean megakaryocyte diameter (µm) |                    |                    |
| Femoral marrow                | 24 ± 1.3          | 38 ± 1.0          |
| Spleen                        | 41 ± 1.9          | 38 ± 1.1          |

Megakaryocyte counts (mean ± SEM) were determined on duplicate cytospin preparations of 2 × 10⁶ femoral marrow or spleen cells from five separate mice of each type. Megakaryocyte diameters are the mean ± SEM from measurements on 25 megakaryocytes per mouse in the same preparations.

* P = .02.
dromes have, like gunmetal, abnormal membrane organization in megakaryocytes and platelets,\textsuperscript{35-37} or an increase in numbers of bone marrow megakaryocytes.\textsuperscript{39} Gunmetal is similar to the gray platelet syndrome in that both have macrothrombocytopenia, deficiencies in alpha-granule proteins, and appearance of alpha-granule proteins in plasma. However, inheritance is by a dominant mode in one kindred of gray platelet patients,\textsuperscript{39} whereas it is recessive in gunmetal. Also, unlike in gunmetal, megakaryocyte number is normal in gray platelet syndrome patients. Another recently described\textsuperscript{40} inherited thrombocytopenia quite closely resembles that of the gunmetal mouse in that it is accompanied by an increase in numbers of bone marrow megakaryocytes, a reduced rate of platelet production, and abnormalities in platelet alpha granules. The development and use of diagnostic molecular probes specific for each mutation should resolve whether any of these or similar syndromes are homologous to that of the gunmetal mouse.

Inherited thrombocytopenia due to decreased rates of platelet synthesis is likely to be relatively common in humans.\textsuperscript{7,8} Animal models such as gunmetal with abnormal platelet synthesis should prove valuable in understanding these syndromes and in providing useful tools for exploring the role of genes that regulate thrombopoiesis.

REFERENCES


Table 4. Megakaryocyte DNA Distributions in Normal (gm/+ and Gunmetal (gm/gm) Mice

<table>
<thead>
<tr>
<th>DNA Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Mice</td>
</tr>
<tr>
<td>gm/+</td>
</tr>
<tr>
<td>gm/gm</td>
</tr>
</tbody>
</table>

DNA distributions were obtained from analysis of 611 to 1,913 > 8N cells for each mouse. Values represent mean ± SEM.
* P = .001.
† P = .01.


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