The Wistar Furth Rat: An Animal Model of Hereditary Macrothrombocytopenia


The mechanisms that determine and regulate platelet size are unknown. By phase microscopy, we observed that Wistar Furth (WF) rats had macrothrombocytopenia. In this study, we have characterized and compared platelets and megakaryocytes of WF rats with those of Wistar, Long-Evans hooded (LE), and Sprague-Dawley rats. In addition, we have examined the mode of inheritance of this WF rat platelet abnormality. The average platelet count of WF rats was only one-third that of the other three rat strains. In contrast, the mean platelet volume (MPV) of adult WF rats was twice that of the other rat strains; however, the average megakaryocyte diameter and DNA content distribution of WF rats were not significantly different from those of LE rats. The average megakaryocyte concentration was 30% lower in the WF strain compared with that of LE rats. Mazelike membrane formations were observed in WF platelets and megakaryocytes by electron microscopy. Reciprocal crosses of WF and LE rats resulted in offspring with MPVs and platelet counts like those of LE rats, indicating that the macrothrombocytopenic trait is recessive in its inheritance. Reciprocal marrow transplants between the WF and LE strains resulted in MPVs like those of the donor strain, demonstrating that the macrothrombocytopenia is an intrinsic marrow abnormality of the WF strain. Splenectomy did not alter the MPV of WF rats. The response of WF megakaryocytes and platelets to severe, acute thrombocytopenia was similar to that of LE rats except that the shift to higher megakaryocyte DNA contents was muted and platelet recovery was slower in the WF rats. In summary, the WF rat has a hereditary macrothrombocytopenia that is recessive in nature and not due to differences in megakaryocyte size or DNA content. These results suggest that the macrothrombocytopenia of WF rats results from the formation of fewer platelets per megakaryocyte, possibly resulting from a qualitative or quantitative defect in some component necessary for proper subdivision of megakaryocyte cytoplasm into platelets.

PLATELET SIZE varies among species but is relatively constant among normal individuals of a given species1; however, the mechanisms that determine and regulate platelet size are unknown. Platelet size is increased in thrombocytopenias due to increased platelet destruction2-5 or during recovery from transient marrow suppression.6,7 In addition, patients with certain platelet disorders and anomalies such as Bernard-Soulier syndrome,8 May-Hegglin anomaly,9 and other less well defined thrombocytopenias10-17 as well as individuals of certain ethnic groups18-22 have abnormally large platelets. In contrast, platelet size is decreased in patients with Wiskott-Aldrich syndrome.23,24 Yet, no small laboratory animal models with hereditary abnormalities of platelet size have been reported. By phase-contrast microscopy, we serendipitously observed that Wistar Furth (WF) rats had fewer, but substantially larger platelets than those of other laboratory rat strains. In this study, we have characterized and compared platelets and megakaryocytes of WF rats with those of other rat strains. In addition, we have examined the mode of inheritance of the macrothrombocytopenia of the WF rat.

MATERIALS AND METHODS

WF, Wistar, Sprague-Dawley and Long-Evans hooded (LE) rats were purchased from Harlan Industries, Inc (Indianapolis, IN). Splenectomized WF and LE rats were purchased from the same supplier. LE rats were usually used as reference controls because of the considerable baseline data we have accumulated on this strain and because their coat color allowed them to be readily distinguished from WF rats. Platelet counts were determined by phase microscopy. Other blood cell parameters were measured with a Cell-Dyne 900 (Sequoia-Turner) automated cell counter.

Platelet volume measurements. Mean platelet volumes (MPVs) were determined with a microcomputerized Elzone electronic particle counter (Particle Data Laboratories, Inc., Elmhurst, IL) with a 48-μm-diameter orifice and a 128-channel analyzer. The instrument was calibrated with 2.02-μm-diameter latex beads before each use. Data were accumulated on each platelet sample until a count of 4,000 was achieved in the peak channel. The samples were diluted such that the coincidence level was less than 1%. The volume distributions were analyzed as log-normal distributions. The data were expressed as MPVs. For platelet size analysis, blood was collected into Na2EDTA at a final concentration of 12.5 mmol/L. To ensure collection of the large platelets present in blood of WF rats, the platelets were separated from other blood cells on self-generated Percoll density gradients rather than by the customary differential centrifugation. Percoll density medium (Pharmacia, Inc., Piscataway, NJ) was made isotonic for rat blood with 10x Dulbecco’s phosphate-buffered saline (PBS) without calcium and magnesium, and a 40% concentration of this medium was made by dilution with an isotonic solution of the same buffer. The medium also contained 5 mmol/L Na2EDTA, which was necessary to prevent platelet aggregation in the gradients. Continuous-density gradients were generated by centrifugation of 9 mL of the diluted Percoll medium at 20,000 g for 20 minutes. One-milliliter samples of blood were diluted 1:1 with Dulbecco’s PBS, placed on top of the density gradients, and centrifuged at 1,000 g for ten minutes. The portion of the cell suspension containing platelets was removed and platelet size...
analyzed. Calculated platelet recoveries from these gradients averaged 100% ± 23% (1 SD) based on analysis of 14 gradients. Recovery of WF platelets was not different than that for the rat strains with smaller platelets. Platelet size analysis was performed two to six hours after blood collection on the basis of the observation by Levin and Bessman19 that the size of human platelets in EDTA-anticoagulated blood was stable over this time period.

Platelet survival. WF and LE platelets were isolated from 60 mL of blood collected in acid citrate and protargol (PGE7, 8 

µmol/L) in self-generated Percoll gradients starting with 40% Percoll as just described but using rat plasma and CATCH medium20 as the dilution medium. Platelets were washed, suspended in Hanks' buffered saline solution (BSS), and labeled with 1 mCi of 111In oxide (Amersham Corp, Arlington Heights, IL) for 30 minutes at room temperature. The platelets were then pelleted and resuspended in 1:1 plasma–Hank's BSS solution and infused into recipient rats through a tail vein.

Twenty-microliter samples of blood were collected from the same rats under methoxyflurane anesthesia by venipuncture of a peripheral vein. The platelets were then pelleted and resuspended in 3 mL of ice-cold Dulbecco's modified Eagle's medium21 as the square root of the product of two measurements made at right angles.

Megakaryocyte DNA distribution. The DNA distribution of megakaryocytes in unfractonated marrow was determined as previously described.21

Bone marrow megakaryocyte concentration. The average number of megakaryocytes per high-power field (HPF, 500× magnification) was determined on sternal bone marrow sections that were fixed in B-5 fixative, decalcified, and stained with hematoxylin and eosin. One entire longitudinal section was evaluated for each rat. Megakaryocytes were recognized by their large size; large lobulated nuclei with thick, clumped, deeply basophilic staining; and usually abundant cytoplasm. Megakaryocyte concentrations of animals with increased average megakaryocyte diameter as a consequence of acute induction of thrombocytopenia were corrected for multiple counting errors22 by dividing the number of megakaryocytes per HPF by the quotient of the average megakaryocyte diameter of the individual thrombocytopenic rat divided by the average megakaryocyte diameter of untreated control rats of the same strain.

Megakaryocyte concentrations were also estimated in a small number of rats by counting the number of acetylcholinesterase-positive (AChE) cells in femoral marrow suspension. Femurs were removed after rats were killed by exsanguination under methoxyflurane anesthesia and subjected to five minutes in 1.25% B-S fixative, decalcified, and stained with hematoxylin and eosin. The DNA distribution of megakaryocytes was determined on sternal bone marrow sections from each rat divided by the average megakaryocyte diameter of the individual thrombocytopenic rat divided by the average megakaryocyte diameter of untreated control rats of the same strain. Megakaryocyte concentrations were also estimated in a small number of rats by counting the number of acetylcholinesterase-positive (AChE) cells in femoral marrow suspension. Femurs were removed after rats were killed by exsanguination under methoxyflurane anesthesia and subjected to five minutes in 1.25% B-S fixative, decalcified, and stained with hematoxylin and eosin. The DNA distribution of megakaryocytes was determined on sternal bone marrow sections from each rat divided by the average megakaryocyte diameter of the individual thrombocytopenic rat divided by the average megakaryocyte diameter of untreated control rats of the same strain.

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Electron microscopy. Platelets from blood collected in acid citrate and 8 µmol/L PGE7, were isolated in Percoll gradients as described earlier (except that 8 µmol/L PGE7 was substituted for EDTA and Hanks' BSS was used to dilute the Percoll), washed once in Hanks' BSS, fixed in 2% glutaraldehyde in 0.1 mol/L cacodylate buffer at 4°C overnight, and postfixed with osmium tetroxide for one hour. Acid citrate and PGE7 were used for platelet collection here instead of EDTA to preserve better the platelet ultrastructure. A small number of platelet aggregates were detected after centrifugation in the gradients but were considered a necessary compromise to achieve improved morphology over that seen with EDTA collection. Small pieces of femoral marrow were fixed in 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer. The samples were dehydrated in ethanol and embedded in resin. Sections were cut and stained for 20 minutes in aqueous uranyl acetate and then 20 minutes in Reynolds' lead stain. In some cases, ruthenium red26 was added during glutaraldehyde fixation of platelets to examine whether internal membrane complexes were surface connected.

Labeling of surface glycoproteins. Platelet surface glycoproteins were labeled with the periodate/NaBH4 procedure,27 which labels mainly sialic acid residues. The platelets (109/mL) were suspended in 0.001 mol/L EDTA, 0.01 mol/L HEPES, and 0.15 mol/L NaCl, pH 7.6. Sodium m-periodate was added at a concentration of 1 mmol/L and the platelets incubated for ten minutes at 4°C in the dark. After washing and resuspension, NaBH4 (New England Nuclear) was added (1 mCi/109 platelets) and the suspension incubated for ten minutes at room temperature. The platelets were washed and solubilized for gel electrophoresis by boiling for five minutes in one-third vol of gel sample buffer containing 0.125 mol/L Tris-HCl, pH 6.8, 4% glycerol, 8% sodium dodecyl sulfate (SDS), 160 mmol/L dithiothreitol, and 0.01% bromphenol blue. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 7.5% acrylamide by using the discontinuous buffer system of Laemmli.28 Proteins were stained with Coomassie blue and the
An exposure of given whole-body radiation at other rat strains (Table calf serum approximately 20 hours after irradiation. LE recipients while 1,400 R was necessary for marrow suppression in LE recipients.

-gels with EN3HANCE (New England Nuclear).-tritium detected by fluorography at -70°C after impregnation of the gels with EN3HANCE (New England Nuclear).

Reciprocal bone marrow transplantation. Recipient rats were given whole-body radiation at the rate of 164 R/min with a 137Cs source. An exposure of 1,050 or 1,200 R was used for WF recipients, while 1,400 R was necessary for marrow suppression in LE recipients. WF recipients were given either 6 x 10^7 LE or 7 x 10^7 WF rat marrow cells IV in 1 mL of α medium supplemented with 5% fetal calf serum approximately 20 hours after irradiation. LE recipients received either 4.3 x 10^7 WF or 4.6 x 10^7 LE rat marrow cells IV in 1 mL of the same medium at a similar time after irradiation. WF donors in this latter case were young female rats weighing 47 to 63 g. These young rats were chosen because their marrow was more easily suspended than that of older WF rats. Platelet size measurements were made as described earlier after the platelet counts had recovered and stabilized.

Induction of acute, severe thrombocytopenia. Acute, severe thrombocytopenia was induced by intraperitoneal injection of rabbit-antirat platelet antiserum prepared as previously described.

RESULTS

Hematocrit values of WF rats were 6% to 7% lower than those of Wistar and LE rats (Table 1); however, platelet counts of this strain were only about one third of that of Wistar, LE, and Sprague-Dawley rats (Table 1), but platelets of WF rats were on average much larger and showed more size heterogeneity than those of other rat strains (Fig 1). The MPVs of adult WF rats were about twice those of the other rat strains (Table 1). There was, however, a relationship between the age of WF rats and MPV, with 3-week-old rats having an average MPV of 6 fL (Fig 2). The MPV gradually increased to an average of 8.3 fL at 18 to 22 weeks.

Individual older WF rats showed considerable MPV variation; however, the platelet mass (platelet count x MPV) per unit volume of blood remained relatively constant over the age range studied. There was little variation in MPVs of LE rat platelets with age.

Smaller MPVs (found in younger WF rats) were accompanied by higher platelet counts (Fig 3). Platelet counts decreased as the MPV increased (Fig 4) so that the circulating platelet mass per volume of blood (MPV x platelet count) remained relatively constant.

Examination of marrow sections revealed that megakaryocyte concentrations were about 30% lower in WF rats compared with the LE strain whereas the average megakaryocyte diameter was not significantly different for the two strains (Table 2). Megakaryocyte concentration was also examined by light microscopic counting of AChE-positive cells in suspensions of femoral marrow with similar results. WF rat marrow contained 47,500 ± 12,874 large AChE cells (±13 μm in diameter) and 5,833 ± 3,192 small AChE cells (<13 μm in diameter) per femur, whereas the values for LE rat marrow were 85,000 ± 7,935 and 11,667 ± 3,333, respectively when marrows of four 2½-month-old rats of each strain were examined. The somewhat lower megakaryocyte concentrations of WF compared with LE rats in marrow cell suspensions v intact marrow sections may be related to our observations that WF marrow cells were more difficult to

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>No. of Rats</th>
<th>Hct (%)</th>
<th>Platelet Counts (x 10^3/μL)</th>
<th>MPV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WF</td>
<td>17</td>
<td>41.9 ± 1.2*</td>
<td>308 ± 51</td>
<td>8.5 ± 1.4 (37)†</td>
</tr>
<tr>
<td>Wistar</td>
<td>6</td>
<td>44.4 ± 0.5 (5)</td>
<td>1,086 ± 68</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>LE</td>
<td>17</td>
<td>45.0 ± 2.0</td>
<td>896 ± 128</td>
<td>4.2 ± 0.3 (30)</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>7</td>
<td>—</td>
<td>926 ± 82</td>
<td>4.1 ± 0.2</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of rats studied when the number was different from that indicated in column 2. Average platelet counts and MPVs of WF rats were significantly different from those of the other rat strains (P < .000001). *Mean ± 1 SD. †MPVs in this Table were from adult animals ≥10 weeks of age.

![Fig 1](Representative size distribution of WF (dashed line) v LE (solid line) rat platelets. Each curve represents a single adult rat. Four thousand platelets are in the peak channel.)

![Fig 2](Relationship between the ages of WF and LE rats and the MPVs. Each point represents the MPV of an individual rat. A small number of rats was studied at more than one time interval. Open circles, WF rats; closed circles, LE rats.)
sustain than those of LE rats and, therefore, perhaps a lower proportion of WF compared with LE megakaryocytes were recovered after marrow suspension.

Based on combined ultrastructural and microspectrophotometric studies, Penington et al\(^3\) proposed that there was a relationship between platelet size and megakaryocyte DNA content. If this were true for WF rats, then megakaryocytes of WF rats, because they produce larger platelets, should have a different DNA content distribution compared with LE rats. Data presented in Table 3 indicate that megakaryocyte DNA distributions for these two rat strains were not significantly different.

**Megakaryocyte maturation time.** We considered the possibility that the production of large platelets in WF rats may result from accelerated megakaryocyte maturation like that seen after induction of acute, severe thrombocytopenia.\(^3\) If this were the case, then the WF megakaryocyte turnover rate should be more rapid than that of other rat strains. Megakaryocyte turnover time was estimated by examining the rate at which labeled precursors replaced unlabeled recognizable megakaryocytes after a single injection of \(^3\)H-thymidine. The shape of the \(^3\)H-thymidine labeling index curve for WF megakaryocytes was not different from that of LE rats (Fig 5), which suggests that the megakaryocyte turnover time for WF rats is not shorter than that of other rat strains.

**Platelet survival time.** The circulation times of platelets of WF and LE rats were quite similar, as depicted in Fig 6. The initial platelet recovery (proportion of injected radioactivity circulating one hour later) averaged 44\% ± 8\% for LE platelets and 59\% ± 4\% for WF platelets (seven rats). Platelet recoveries were higher in a second experiment (LE platelets into WF rats, 63\% ± 8\% [four rats]; WF platelets into LE rats, 59\% ± 6\% [three rats]; WF platelets into WF rats, 66\% ± 8\% [seven rats]), but the subsequent platelet disappearance was slightly more rapid. Nevertheless, the similarities of initial platelet recoveries among the experimental groups in these two experiments suggest that the splenic platelet pool size in WF and LE rats is similar.

**Inheritance pattern of WF macrothrombocytopenia.** We next examined the mode of inheritance of the large platelet phenotype of WF rats. All F\(_1\) offspring of reciprocal crosses between WF and LE rats had MPVs like LE rats (Table 4), indicating that inheritance of the large platelet phenotype of WF rats is recessive to that of LE rats. All F\(_1\) offspring had the black and white coat color of the LE strain. We next backcrossed male F\(_1\) offspring with female WF rats to examine whether a single or multiple alleles were responsible for the large platelet phenotype. If a single allele is involved, then the ratio of offspring with the large platelet phenotype to those with the normal MPV should be 1:1. Such a 1:1 relationship was observed (Table 5). The platelet size of

### Table 2. Marrow Megakaryocyte Values

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>No. of Rats</th>
<th>Megakaryocyte Concentration/HPF*</th>
<th>Average Megakaryocyte Diameter (µm)†</th>
</tr>
</thead>
</table>
| WF         | 7           | 4.5 ± 0.5I8 \(P < .001\)         | 18.4 ± 0.5.Serialization of WF megakaryocytes was not different from that of LE rats (Fig 5), which suggests that the megakaryocyte turnover time for WF rats is not shorter than that of other rat strains. Part 1. Relationship between the ages of WF rats and platelet counts. Each point represents the platelet count of an individual rat (data from the same rats represented in Fig 2).

Fig 3. Relationship between the ages of WF rats and platelet counts. Each point represents the platelet count of an individual rat (data from the same rats represented in Fig 2).

Fig 4. Inverse relationship between MPV and platelet count in WF rats. Each point represents one rat (derived from data in Figs 2 and 3).
Table 4. MPVs of F₁, Hybrids of Crosses Between WF and LE Rats

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>No. of Rats</th>
<th>Sex</th>
<th>MPV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WF</td>
<td>15</td>
<td>M</td>
<td>8.1 ± 1.4*</td>
</tr>
<tr>
<td>LE</td>
<td>13</td>
<td>M</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>LE female x WF male</td>
<td>3</td>
<td>M</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>LE female x LE male</td>
<td>3</td>
<td>F</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>WF female x LE male</td>
<td>3</td>
<td>M</td>
<td>4.1 ± 0.0</td>
</tr>
<tr>
<td>WF female x WF male</td>
<td>3</td>
<td>F</td>
<td>4.3 ± 0.4</td>
</tr>
</tbody>
</table>

The MPV of WF rats was significantly larger than those of LE rats or offspring of crosses of these two rat strains (P < .000001).

*Mean ± 1 SD.

The cytoplasm of WF rat megakaryocytes often contained membrane complexes and disordered demarcation membrane arrangements (Fig 8).

Surface glycoproteins of platelets. No differences were detected between the H-sodium borohydride labeling profiles of WF and LE platelet surface glycoproteins after mild periodate treatment (Fig 9).

Reciprocal marrow transplantation. To examine whether the large platelet phenotype of WF rats was due to an intrinsic abnormality of the megakaryocytic series or to some more general metabolic characteristic of WF rats, reciprocal marrow transplants were performed between the WF and LE strains. The MPV after marrow reconstitution was like that of the marrow donor (Table 6), indicating that the large platelet phenotype is an intrinsic characteristic of WF megakaryocytes.

Is there a relationship between the spleen and macrothrombocytopenia of WF rats? von Behrens reported that mild splenomegaly was present in individuals with Mediterranean macrothrombocytopenia and that the MPV directly correlated with spleen size in individuals with this trait; however, splenectomy did not affect the MPV in three such patients. We examined spleen weights and MPVs after splenectomy in WF rats to ascertain whether splenomegaly was present and whether splenectomy affected the MPV. Spleen weights in 3-month-old WF and LE rats were not significantly different: 655 ± 57 mg (eight rats) and 716 ± 115 mg (seven rats), respectively; however, because WF rats are smaller than LE (in this case, 270 ± 17 g vs 358 ± 36 g), the average spleen weight expressed in milligrams per 100 grams of body weight was significantly higher (P < .005) in WF compared with LE rats: 243 ± 17 g vs 199 ± 23.

More to the point, MPVs of splenectomized WF rats (studied 7 weeks postsplenectomy) were not significantly different from those of intact WF rats (Table 7); however, platelet counts of splenectomized WF rats were slightly

Table 5. Platelet Phenotypes After Backcross of (WF x LE) F₁, Males With WF Females

<table>
<thead>
<tr>
<th>Platelet Size</th>
<th>Phenotype*</th>
<th>No. of Offspring</th>
<th>Platelet Count (x 10⁹/µL)</th>
<th>MPV (fl)</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>24</td>
<td>785 ± 114†</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Large</td>
<td>21</td>
<td>322 ± 73</td>
<td>6.9 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Mean platelet counts and MPVs of the two groups were significantly different (P < .000001).

†Mean ± 1 SD.
Fig 7. Ultrastructure of WF platelets that illustrates abnormal membrane complexes (indicated by arrows). The platelet on the left shows a membrane complex region that resembles Swiss cheese, whereas the platelet on the right has a more dense membrane complex giving a spaghetti-like appearance. Lower panels are enlargements of the membrane complexes indicated by arrows in the upper panel.

Fig 8. (A) Example of membrane complexes often observed in the cytoplasm of mature WF rat megakaryocytes. (B) Typical ultrastructure of cytoplasm of mature LE rat megakaryocytes.

higher than those of intact WF controls. MPVs of splenectomized LE rats were slightly larger than those of intact LE rats, while platelet counts of these two groups were not significantly different.

Comparison of the effects of acute, severe thrombocytopenia on MPV and megakaryocytes of WF and LE rats. Acute, severe thrombocytopenia was induced by intraperitoneal injection of platelet antiserum (APS). In one experiment, serial platelet counts were determined on the same rats through day 13 to study the pattern of platelet recovery. In a second experiment, rats were killed at intervals to evaluate platelet count, MPV, and megakaryocytes. Platelet counts of both strains were reduced to <5% of baseline at 24 hours. Platelets of WF rats recovered more slowly and reached pretreatment levels one day later than those of LE rats and showed a lesser degree of rebound thrombocytosis compared with the LE strain (Fig 10).

MPV of WF rats was increased an average of 62% on days 2 and 3 and by 32% on day 5 after APS (Table 8). The MPV of LE rats was increased by an average of 84% on day 2 and 42% on day 3 and had returned to the normal range on day 5.

As anticipated, the megakaryocyte DNA content distribution of LE rats shifted to the right, with 32N cells forming the major DNA class on days 2 and 3 after APS along with a substantial number of 64N cells and a small group of 128 N
Table 6. Platelet Number and Size 29 to 147 Days After Reciprocal Marrow Transplantations Between WF and LE Rats

<table>
<thead>
<tr>
<th>Marrow Donor</th>
<th>Marrow Recipient</th>
<th>No. of Recipients</th>
<th>Platelet Count (x 10^12/μL)</th>
<th>MPV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>WF</td>
<td>8</td>
<td>697 ± 79*</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>LE</td>
<td>LE</td>
<td>6</td>
<td>719 ± 104</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>WF</td>
<td>LE</td>
<td>6</td>
<td>270 ± 61</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td>WF</td>
<td>WF</td>
<td>9</td>
<td>244 ± 37</td>
<td>8.7 ± 0.7</td>
</tr>
</tbody>
</table>

Mean platelet counts and MPVs of the two groups of marrow recipients that received transplants from the same marrow donor strain were not significantly different; however, platelet counts and MPVs in recipients that received transplants with marrow from different donor strains were significantly different at a minimum of P < .001.

*Mean ± 1 SD.

Table 7. Splenectomy Does Not Affect MPV of WF Rats

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>No. of Rats</th>
<th>Platelet Count (x 10^12/μL)</th>
<th>MPV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WF</td>
<td>Splenectomized 8</td>
<td>364 ± 37*†</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Intact 7</td>
<td>304 ± 42</td>
<td>7.2 ± 0.25</td>
</tr>
<tr>
<td>LE</td>
<td>Splenectomized 4</td>
<td>861 ± 80</td>
<td>4.4 ± 0.2‡</td>
</tr>
<tr>
<td></td>
<td>Intact 5</td>
<td>986 ± 143</td>
<td>4.0 ± 0.25</td>
</tr>
</tbody>
</table>

Splenectomies were performed at 3 weeks of age. Platelets were studied at 10 weeks of age.

*Mean ± 1 SD.

†Platelet counts of splenectomized WF rats were significantly higher (P < .02) than those of intact WF rats. Platelet counts of intact and splenectomized LE rats were not significantly different.

‡MPVs of splenectomized LE rats were significantly higher (P < .02) than those of intact LE rats.

DISCUSSION

Hereditary clinical abnormalities in platelet size have been observed including patients with large MPVs as well as...
those with smaller mean platelet size. To our knowledge, the macrothrombocytopenia in WF rats described here represents the first such abnormality in small laboratory animals. We detected large platelet size and reduced platelet number in WF rats when asked to perform platelet counts for two immunology colleagues. Although platelet counts of WF rats were only about one third lower than that of the other rat strains, the approximately 30% lower marrow megakaryocyte concentration but similar megakaryocyte size in WF compared with LE rats. The similar average megakaryocyte diameters of these two strains suggested that DNA content distributions of WF megakaryocytes would resemble those of LE rats, as was the case.

The platelet size of WF x LE crosses was like that of the LE strain, thus indicating that the macrothrombocytopenia of WF rats was recessive to the platelet size of LE rats.

Platelet size analyses of offspring of backcrosses of these F1 hybrids with WF females yielded an approximately 1:1 ratio of macrothrombocytopenic to normal platelet phenotype animals, which suggested that a difference in a single allele is responsible for the macrothrombocytopenia of WF rats.

The MPV of recipients after reciprocal marrow transplants between WF and LE rats was that of the donor strain, indicating that platelet size was a characteristic of the donor marrow. The simplest explanation of these transplant experiments is that the macrothrombocytopenia of WF rats results from an intrinsic abnormality in their megakaryocytes; however, the age-related increase in MPV suggests that the megakaryocyte abnormality of WF rats is permissive in

Table 8. Comparison of Platelet Counts and MPVs in WF and LE Rats After APS Injection

<table>
<thead>
<tr>
<th>Time After APS Injection (d)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Rats</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Platelet count (× 10^9/μL)</td>
<td>271 ± 50*</td>
<td>10 ± 1</td>
<td>20 ± 4</td>
<td>40 ± 24</td>
<td>220 ± 21</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>7.3 ± 0.4</td>
<td>†</td>
<td>11.8 ± 1.4†</td>
<td>11.8 ± 0.3§</td>
<td>9.6 ± 0.8]</td>
</tr>
<tr>
<td><strong>LE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Rats</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Platelet count (× 10^9/μL)</td>
<td>878 ± 107</td>
<td>16 ± 4</td>
<td>86 ± 50</td>
<td>285 ± 107</td>
<td>1,166 ± 341</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>4.3 ± 0.2</td>
<td>—</td>
<td>7.9 ± 1.7‖</td>
<td>6.1 ± 0.5†</td>
<td>4.6 ± 0.4</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD.
†The low number of platelets on day 1 after APS injection did not allow adequate resolution of the platelet size distribution from background noise.
‡Significantly different from untreated controls (day 0), P < .005.
§P < .001.
‖P < .02.

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nature and that some age-related metabolic trait affects the MPV of these rats.

Expression of platelet size only as MPV can be misleading because it does not reveal that platelet size is actually more heterogeneous in WF rats (Fig 1), as also has been noted by Paulus et al32 in patients with macrothrombocytopenia.

Normal platelet survival and response to aggregating agents suggest that the large WF platelets behave and function normally. The surface glycoprotein profile of WF platelets as revealed by periodate-3H-sodium borohydride labeling was also not different from that of LE platelets and resembled that described in earlier reports of rat platelets.23,24 Ultrastructural studies, however, revealed that abnormal membrane formations were present in WF platelets and megakaryocytes, like those described in patients with macrothrombocytopenia,10,13,16,35 and in rats with experimentally induced thrombocytopenia.36

Of the clinical macrothrombocytopenias described, that of the WF rat seems to most resemble Mediterranean macrothrombocytopenia in which normal platelet survival19,37 and function14 have been reported with platelets containing mazelike membrane formations revealed by ultrastructural analysis;2 however, the age-related increase in MPV we observed in WF rats has not been detected in patients with Mediterranean macrothrombocytopenia.16 Although extensive histopathologic studies of WF rats have not been performed, no histological evidence of renal disease and deafness like that associated with macrothrombocytopenia in patients described by Epstein et al10 and others12,13 was observed. In addition, Döhle bodies were not observed in WF granulocytes, which suggests that this rat model is not analogous to the May-Hegglin anomaly in humans.

The WF rat seems to provide a tool to examine the process of platelet size determination. In this study, we explored the possibility that the large platelet size of WF rats may be associated with accelerated megakaryocyte maturation; however, the nearly identical 3H-thymidine labeling curves indicate that megakaryocyte turnover is not more rapid in WF rats. On the basis of combined ultrastructural and microspectrophotometric studies of rat megakaryocytes and platelets, Penington et al10,37 concluded that platelet size was related to the DNA content of the megakaryocytes from which they were derived. In their initial analysis, Penington and Streetfield10 reported that in comparison to 8N cells of comparable maturity, the cytoplasm of 32N mature megakaryocytes was less dense and homogeneous. From the differences in the patterns of the demarcation membrane system between these two megakaryocyte DNA classes, they hypothesized that 32N cells produce larger platelets than 8N cells. In a subsequent ultrastructural comparison of megakaryocyte cytoplasm with density-fractionated platelets, this group30 found that the morphology of the most dense platelets, which were the largest in size, resembled that of the cytoplasm of mature 8N megakaryocytes, whereas the ultrastructure of the lightest platelets, which were the smallest, resembled that of 32N megakaryocytes. From these observations, they then hypothesized the opposite relationship between megakaryocyte DNA content and platelet size, namely, that platelet size was inversely related to the megakaryocyte DNA content. The large platelets of WF rats are not related to altered megakaryocyte DNA content because the DNA content distributions of WF and LE rat megakaryocytes were not significantly different. Another mechanism of platelet size determination proposed by Trowbridge et al38 and Martin et al39 is that platelet size is determined by lung capillary diameter. The platelet size analyses after reciprocal marrow transplants described here do not support this hypothesis.

The spleen may sometimes affect platelet size. For instance, Corash et al40 found that the small MPV associated with Wiskott-Aldrich syndrome was returned to the normal range by splenectomy and that the normal MPV was maintained even when thrombocytopenia again developed post-splenectomy. In Mediterranean macrothrombocytopenia, von Behrens14 found an association between spleen size and MPV; however, the MPV was not affected by splenectomy. Our results with the WF rat bear similarities to those of individuals with Mediterranean macrothrombocytopenia in that spleen size per unit of body weight is slightly larger than that of LE rats and that the MPV was not affected by splenectomy. Initial platelet recovery in the platelet survival experiments was similar in WF and LE rats, which suggests that the splenic pool size was not different in these two strains. Taken together, these results suggest that the spleen does not contribute to the macrothrombocytopenia of WF rats.

The response of WF megakaryocytes to severe, acute thrombocytopenia showed several similarities to those of LE rats. The MPV was increased in both strains on days 2 and 3 as expected.41 Megakaryocyte number42,23 and diameter40,44 were also increased in both strains during this time. Also, the DNA content distributions of both WF and LE megakaryocytes showed an increased proportion of >16N cells after induction of thrombocytopenia; however, rather than showing a shift from 16N to 32N as the major megakaryocyte DNA class on days 2 and 3 as seen in LE rats in this and previous studies of LE31 and Sprague-Dawley rats45,46 and C57/BL mice,41 in WF rats 16N remained the major DNA class. This suggests either (a) that WF megakaryocytes are less responsive to a thrombopoietic stimulus, (b) that the increase in thrombopoietic-stimulating factor(s) in WF rats in response to thrombocytopenia is muted, or (c) that because the MPV is larger in the WF strain the platelet count in WF rats was not reduced low enough to elicit the same thrombopoietic response. This last possibility seems least likely because Corash et al40 have observed that in mice a reduction in platelets to 15% of untreated control values still produced a shift to the right in megakaryocyte DNA content distributions so that 32N became the major DNA class. The average platelet count of the thrombocytopenic WF rats 24 hours after APS administration was 3.7% of that of untreated controls. The fact that the narrow megakaryocyte concentration was increased to a similar degree in both WF and LE rats suggests that the thrombopoietic stimulus in response to thrombocytopenia is similar in both rat strains. Therefore, of the three possibilities considered, the most likely is that WF megakaryocytes are less responsive to thrombopoietic-stimulating factor. The slower platelet
recovery rate and lesser degree of rebound thrombocytosis in WF rats is consistent with this conclusion.

In summary, the WF rat provides a model of hereditary macrothrombocytopenia that should be useful in studying the determination and regulation of platelet size during megakaryocyte differentiation.

ACKNOWLEDGMENT

We wish to acknowledge Drs Bruce Veit and Otto Schofer as the immunology colleagues who asked us to perform platelet counts on WF rats, which then resulted in our recognizing this hereditary macrothrombocytopenia. We are indebted to Dr Janine Breton-Gorus for providing expertise in the evaluation of the ultrastructure of megakaryocytes and platelets. We are grateful for the technical expertise of Graydon Nelson for flow cytometry. We also wish to acknowledge the advice of Dr Mark Costlow in performing the platelet size analyses. The word processing skills of Peggy Vandiveer are also deeply appreciated.

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The Wistar Furth rat: an animal model of hereditary macrothrombocytopenia

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