Platelet Storage Pool Deficiency in Mouse Pigment Mutations Associated With Seven Distinct Genetic Loci

By Edward K. Novak, Sek-Wen Hui, and Richard T. Swank

Seven mouse pigment mutants, which have alterations at distinct genes, are known to have a defect in kidney lysosomal enzyme secretion. Two of these, beige and pale ear, have a bleeding abnormality associated with a deficiency in the number of platelet dense granules. In the present study, five other mutants with defective lysosomal enzyme secretion—pearl, pallid, light ear, maroon, and ruby-eye—were likewise found to have abnormally prolonged bleeding times after experimental injury. Platelet counts were similar to those of normal mice, but the platelet dense granule components serotonin, adenosine diphosphate (ADP) and morphologically identifiable dense granules were markedly reduced in these mutants. The capacity to accumulate exogenous 3H-serotonin in platelets was reduced 2-3-fold. Thrombin-stimulated secretion of 3H-serotonin was slightly decreased in all mutants. However, the seven mutants could be subdivided into three groups based on the degree of secretion of lysosomal enzymes after thrombin stimulation. Thus, all seven mouse pigment mutants have symptoms consistent with platelet storage pool deficiency and may serve as useful animal models for specific types of human platelet storage pool disease. Also, the results emphasize the genetic, morphological, and functional interrelatedness of three organelles: melanosomes, lysosomes, and platelet dense granules.

SEVERAL AUTOSOMAL recessive bleeding disorders in humans that affect platelet dense granule function are known. Among these are storage pool disease (SPD), including Chediak-Higashi syndrome (CHS) and Hermansky-Pudlak syndrome (HPS), Wiskott-Aldrich syndrome and thrombocytopenia absent Radii syndrome. In two diseases (CHS and HPS) the bleeding disorder is accompanied by a dilution in pigmentation.

SPD is characterized by a prolonged bleeding time accompanied by normal platelet counts, decreased platelet dense granule contents and function, and a reduced number of platelet dense granules. Patients with storage pool deficiency often have abnormal structure and/or function of other subcellular organelles, including melanosomes in CHS and HPS, in which patients are tyrosinase-positive albinos, lysosomes in CHS and HPS, and platelet α granules. Weiss and coworkers have recently subclassified SPD based on the relative contents of dense granules and alpha granules. The subclassifications include patients defective in only one, both, or varying degrees of both organelles. These subclassifications are interesting in that these symptoms may reflect either the same genetic lesion on various genetic backgrounds or distinct diseases coded for by different genes.

We and others have been studying several recessively inherited mutations in the mouse that simultaneously affect both melanosomes and lysosomes. Of 31 mouse pigment mutants, 7 abnormally accumulate lysosomal enzymes because of defective kidney lysosomal enzyme secretion. Two of these pigment mutants, the beige mouse (a model for the human Chediak-Higashi syndrome) and the pale ear mouse, which has been proposed to be a model for the Hermansky-Pudlak syndrome, also have a bleeding abnormality accompanied by a deficiency in platelet dense granule contents and in the actual number of platelet dense granules. Furthermore, platelet storage deficiency has been documented in cattle, mink, and cats with Chediak-Higashi symptoms. Also, the fawn hooded rat, an animal with diluted pigmentation, has been shown to have storage pool deficiency caused by a deficiency in platelet dense granules.

We therefore studied bleeding times in other mouse pigment mutants with normal and abnormal rates of lysosomal enzyme secretion. In this article we report that all pigment mutants with altered lysosomal function have prolonged bleeding times and platelet storage deficiency associated with a deficiency in the number of dense granules. They may be useful as animal models for the various subclasses of human platelet storage pool deficiency.

MATERIALS AND METHODS

C57BL/6J (+/+), beige (bg/*/bg*), pale ear (ep/ep), light ear (le/le), pearl (pe/pe), pallid (pa/pa), ruby-eye (ru/ru), maroon (ru-2/*/ru-2*), buff (bf/bf), light (B*/B*), white (Mf*/Mf*), brown-3 (b*/b*), leaden fuzzy (ln fz/ln fz), and viable dominant spotting (w*/w*) were obtained from the Jackson Laboratories, Bar Harbor, ME, and were later bred at the animal facilities of Roswell Park Memorial Institute. All animals were congenic or coisogenic to C57BL/6J. Thus, any mutant abnormality found would, to a very high probability, be assigned directly to the pigment gene rather than to differing background genes. Female mice (3-4 mo old)

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were used for tail bleedings. Male mice (4–6 mo old) were used in all other studies.

**Bleeding Time**

A 2-mm portion of the tip of the tail of unanesthetized mouse was severed using a sharp razor blade. The tail was blotted at 15-sec intervals until bleeding stopped.

**Platelet Collection**

Platelets were collected by a modification of the method of Holland.

**Platelet Counts**

Platelets were counted after dilution in 1% ammonium oxalate according to the method of Brecher and Cronkite.

**Platelet Serotonin Assay**

Platelet pellets were lysed in 1 ml distilled water and assayed for serotonin according to Crosti and Luchelli. Serotonin fluorescence was measured in an Aminco Bowman spectrofluorimeter with activation at 295 nm and emission at 540 nm. Fluorescence was quantitated by comparison to fluorescence of standard serotonin creatine sulfate.

**Platelet Adenine Nucleotides**

Platelet adenine nucleotides were determined by a modification of the method of Holmsen, Storm, and Day.

**Uptake of Serotonin**

Twenty microcuries of ³H-serotonin was added to unlabeled serotonin creatine sulfate, and this mixture was added to platelets in platelet-rich plasma and diluted with 0.85% NaCl to give a 1-ml solution containing 10 µg serotonin and 5 x 10⁴ platelets. Platelets were incubated at various time intervals with serotonin at 37°C. After the incubation was completed, samples were placed in an ice bath, centrifuged, and aliquots of supernatant and pellet removed for scintillation counting.

**Enzyme Assays**

The lysosomal enzymes β-glucuronidase and β-galactosidase were assayed fluorometrically using 4-methylumbelliferyl-β-D-glucuronide and 4-methylumbelliferyl-β-D-galactoside as substrates. The cytosolic enzyme lactate dehydrogenase was assayed spectrophotometrically according to Reeves et al.

**Thrombin-Stimulated Platelet Secretion**

To platelet-rich plasma, 10 µCi ³H-serotonin was added and allowed to incubate 30 min at 37°C. The platelets were washed twice with platelet washing solution (pH 7.5) containing 40 mM NaH₂PO₄, 4.7 mM KH₂PO₄, 103 mM NaCl, 5 mM glucose, and 0.5 mM EDTA. The platelets were resuspended in 1.0 ml Tyrodes solution (minus calcium plus magnesium). Thrombin, at varying concentrations in 0.1 ml, 0.85% NaCl, was added and incubation was continued at 37°C for 3 min in a shaking water bath. The reaction was stopped with 2.5 U hirudin, and the tubes were immersed in the cold. Platelet pellets were removed by centrifugation, and the pellet was resuspended in 1.0 ml Tyrodes solution. The pellet and supernatant were made 1% in Triton X-100. Aliquots were measured for both radioactivity and lysosomal enzyme activity. Under these conditions, there was no increase in extracellular levels (<5% of intracellular) of lactic dehydrogenase.

**Five Microliters of platelet-rich plasma were placed on a carbon-coated grid and allowed to stand 1 min, according to Hui and Costa. The excess fluid was removed with Whatman #4 filter paper. The platelets adsorbed to the grid were dried. The grids were examined, without further staining, in a Siemens 101 microscope at 18,000× magnification. Micrographs of individual platelets were taken at random, and the number of dense granules (0.2 µ or larger) per platelet were counted.**

**Chemicals**

³H-serotonin creatine sulfate was obtained from Amersham (Arlington Heights, IL). Thrombin, hirudin, and adenine nucleotides were obtained from Sigma (St. Louis, MO). All other reagents were obtained from Sigma or Fisher (Fair Lawn, NJ) and were the best grade available.

**RESULTS**

To test if mice with both altered melanosome and lysosome function could also have abnormal platelet function, pigment mutants with lysosome secretion abnormalities were bled experimentally (Table 1), and bleeding times were compared to mice that appeared normal in our original survey. Without exception, mice with combined melanosome and lysosome dysfunction also had prolonged bleeding after experimental injury. Pigmented mice that had normal kidney lysosomal enzyme concentrations did not have prolonged bleeding times and were not investigated further. Interestingly, three pigment mutants, white, brown, and leaden fuzzy, had bleeding times that were significantly shorter than normal. We have not investigated these phenomena further. As can be seen in Table 1, the prolonged bleeding times were not due to a decrease in platelet number.

In order to test whether these mutants, like beige and pale ear, had a deficiency of dense granule contents, platelet serotonin and adenine nucleotides, ATP and ADP, were directly measured. Platelets from light ear and pale ear mice had 4.5-fold less serotonin than normal platelets (Table 2), whereas the platelets from the other pigment mutants had 13–100-fold less serotonin than platelets from normal mice. In platelets isolated from mutant mice, ATP levels are 1.5–2-fold lower, and ADP values are 2.6–6.0-fold lower, than the values found in platelets isolated from normal mice. Normal mouse platelets store 60% of their ADP and ATP in the secretable granular pool, with the remainder being located in the metabolic pool. Thus, most of the deficiency of ATP and ADP in these mutant platelets can be accounted for by a deficiency in the secretable granular pool of adenine nucleotides, which is characteristic of platelet storage pool deficiency. Furthermore, the increased ratio of ATP to ADP in the mutant platelets is also characteristic of storage pool deficiency.
Table 1. Bleeding Times and Platelet Counts in Normal and Mutant Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Chromosome Location</th>
<th>Altered Kidney Lysosomes</th>
<th>Bleeding Time (min) (No. of Mice)</th>
<th>Platelet Counts (Cells/mL × 10^11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J normal</td>
<td>—</td>
<td>—</td>
<td>3.8 ± 0.3 (18)</td>
<td>8.44 ± 0.32</td>
</tr>
<tr>
<td>Beige-J (bg/bg')</td>
<td>13</td>
<td>+</td>
<td>11.6 ± 1.1 (6)*</td>
<td>7.70 ± 0.98</td>
</tr>
<tr>
<td>Pearl (pe/pe)</td>
<td>13</td>
<td>+</td>
<td>&gt;15 (6)*</td>
<td>7.85 ± 0.75</td>
</tr>
<tr>
<td>Pallid (pa/pa)</td>
<td>2</td>
<td>+</td>
<td>&gt;15 (3)*</td>
<td>7.79 ± 0.85</td>
</tr>
<tr>
<td>Maroon (ru-2'/ru-2'')</td>
<td>7</td>
<td>+</td>
<td>14.0 ± 1.0 (5)*</td>
<td>7.46 ± 1.0</td>
</tr>
<tr>
<td>Ruby-eye (ru/ru)</td>
<td>19</td>
<td>+</td>
<td>&gt;15 (4)*</td>
<td>6.92 ± 0.6</td>
</tr>
<tr>
<td>Light ear (le/le)</td>
<td>5</td>
<td>+</td>
<td>13.5 ± 1.5 (6)*</td>
<td>7.27 ± 0.85</td>
</tr>
<tr>
<td>Pale ear (pe/pe)</td>
<td>19</td>
<td>+</td>
<td>14.6 ± 0.4 (7)*</td>
<td>7.67 ± 0.62</td>
</tr>
<tr>
<td>Buff (bf/bf)</td>
<td>5</td>
<td>—</td>
<td>6.8 ± 1.9 (9)</td>
<td>—</td>
</tr>
<tr>
<td>Light (l'/l')</td>
<td>4</td>
<td>—</td>
<td>5.7 ± 0.8 (12)</td>
<td>—</td>
</tr>
<tr>
<td>White (M'M'/M'M')</td>
<td>6</td>
<td>—</td>
<td>1.0 ± 0.2 (6)*</td>
<td>—</td>
</tr>
<tr>
<td>Brown-J (b'/b')</td>
<td>4</td>
<td>—</td>
<td>0.7 ± 0.04 (6)*</td>
<td>—</td>
</tr>
<tr>
<td>Leaden fuzzy (ln fz/ln fz)</td>
<td>4</td>
<td>—</td>
<td>0.7 ± 0.05 (6)*</td>
<td>—</td>
</tr>
<tr>
<td>Viable dominant spotting (w'/w')</td>
<td>5</td>
<td>—</td>
<td>6.9 ± 4.1 (3)</td>
<td>—</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM.

A possible mechanism for decreased platelet dense granule content is an inability to take up and concentrate exogenous molecules. We therefore measured the ability of platelets from normal mice and from pearl, pallid, and light ear mice to take up and accumulate a dense granule component, ^3H-serotonin (Fig. 1). Excess concentrations were selected so that the rate of accumulation, rather than the amount of serotonin, was rate limiting. While normal and mutant platelets initially took up serotonin at similar rates, mutant platelets were saturated more quickly (10 min) than normal platelets, whose rate of incorporation remained linear for 30 min, suggesting a normal transport mechanism but a defective or depressed pool of dense granules. Similar results were obtained when platelets from maroon and ruby-eye mice were measured (not shown).

Therefore, we directly measured the number of dense granules in platelets from normal and mutant mice by electron microscopy. Platelet-rich plasma was placed on a carbon grid and the platelets were allowed to adsorb to the grid and were dried as whole cells. The majority of air-dried platelets appeared to be normally discoid in shape and individually spaced. The platelets were of normal size. Mounted, normal platelets had an even distribution of large dense granules (average diameter 0.2 μm) per platelet, with the majority of platelets having between 5 and 11 large dense granules per platelet (Fig. 2). Each of the mutants, pallid, ruby-eye, pearl, light ear, and maroon, had a vastly decreased number of large dense granules per platelet and a large percentage had no dense granules. Thus, in mutant mice there is an actual deficiency in the number of large dense granules per platelet. When individual platelets are examined (Fig. 3), one finds both a lack of typical large dense granules and occasionally a number of very small granules (average diameter 0.02 μm), suggesting defective formation of dense granules in at least some platelets.

Table 2. Platelet Dense Granule Contents

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Serotonin (μg/10^7 Cells)</th>
<th>ATP (μmole/10^11 Cells)</th>
<th>ADP (μmole/10^11 Cells)</th>
<th>ATP/ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>5.12 ± 0.61 (7)</td>
<td>11.7 ± 0.93 (10)</td>
<td>5.2 ± 0.55</td>
<td>2.25</td>
</tr>
<tr>
<td>Pearl</td>
<td>0.14 ± 0.09 (4)*</td>
<td>6.75 ± 0.92 (10)</td>
<td>1.7 ± 0.41†</td>
<td>3.95</td>
</tr>
<tr>
<td>Pallid</td>
<td>0.04 ± 0.01 (4)*</td>
<td>5.88 ± 0.80 (4)</td>
<td>1.91 ± 0.46†</td>
<td>3.08</td>
</tr>
<tr>
<td>Maroon</td>
<td>0.32 ± 0.06 (4)*</td>
<td>6.27 ± 1.29 (4)</td>
<td>1.45 ± 0.66†</td>
<td>4.32</td>
</tr>
<tr>
<td>Ruby-eye</td>
<td>0.33 ± 0.05 (4)*</td>
<td>7.65 ± 0.53 (4)</td>
<td>0.86 ± 0.16*</td>
<td>8.89</td>
</tr>
<tr>
<td>Light ear</td>
<td>1.12 ± 0.12 (5)*</td>
<td>ND</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>Beige</td>
<td>0.04 ± 0.01 (4)*</td>
<td>5.47 ± 0.70 (8)</td>
<td>2.03 ± 0.44†</td>
<td>2.70</td>
</tr>
<tr>
<td>Pale ear²</td>
<td>1.05 ± 0.16 (8)*</td>
<td>6.43 ± 0.55†</td>
<td>1.89 ± 0.43†</td>
<td>3.40</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM. The number of mice used is in parentheses (ATP and ADP were done on same mice). Significance tests are between normal and individual mutant strains.

ND, not determined.

* p < 0.001.
† p < 0.01.
‡ p < 0.02.
§ p < 0.05.
GENETICS OF STORAGE POOL DEFICIENCY

Fig. 1. Uptake of $^3$H-serotonin by platelets from normal and mutant mice. Concentrations were selected in which serotonin was in excess (10 $\mu$g serotonin/ml). Under these conditions, normal platelets accumulated 1.4%-1.5% of total $^3$H in 1 hr, whereas mutant platelets accumulated 0.6%-0.7% of total radioactivity. The plotted values are the averages of 6 separate platelet preparations from normal mice and of 2 preparations from mutant mice. □ = Normal; ■ = pearl; ● = pallid; ▲ = light ear.

much greater in normal than in mutant platelets (Table 2), with the serotonin/platelet ratio in normal:mutant ranging from 4.5-fold in light ear and pale ear to 100-fold in pallid and beige. An interesting question is whether the few large granules in mutant platelets have normal serotonin concentrations. When serotonin concentration per large dense granule was calculated (from Fig. 2 and Table 2), it was found that large granules in normal platelets had fivefold more serotonin than pallid and pearl granules and only twofold more than ruby-eye and maroon granules. Similar calculations from the previously described pale ear$^7$ and light ear reveal a serotonin:granule ratio that is nearly equal to normal platelets. This suggests that large dense granules from platelets from the mutant mice are able to sequester serotonin in a near-normal fashion.

In order to test whether other organelles are affected in platelets defective in dense granules, we measured dense granule and lysosomal enzyme secretion in thrombin-stimulated platelets. We were interested especially in platelet lysosomal enzyme secretion in order to see whether the lysosomal enzyme secretion defect we first observed in kidneys of pigment mutant mice$^{14,30}$ was present to the same extent in platelets. Dense granule secretion was measured by release of $^3$H-serotonin (Table 3). While normal platelets secreted 91% of their radiolabeled serotonin, the mutant platelets secreted from 61% (pearl) to 77% (pale ear) of their dense granule contents. This slightly lowered secretion is possibly due to a smaller proportion of serotonin in the granular pool in mutant mice. It is known that, in humans with SPD, some exogenous $^3$H-serotonin does not reach the granule and is exposed to and degraded by hydrolytic enzymes, such as monoamine oxidase. Thus, we may not be observing defective secretion but rather normal secretion of the serotonin that reached the granular pool.

Platelets from mutant mice have normal concentrations of the lysosomal enzymes $\beta$-glucuronidase and $\beta$-galactosidase (not shown). When normal and mutant platelets were stimulated with thrombin (Table 3), normal platelets secreted 21% and 22% of these two lysosomal enzymes, respectively. Beige, pearl, and pallid platelets secreted only about 10% of their lysosomal enzyme contents, indicating reduced secretion similar to that found in kidney.$^{16,18}$ However, pale ear and light ear platelets surprisingly responded to thrombin in a different manner, secreting greater than 40% of their lysosomal enzymes. Extracellular lactic dehydrogenase (<5%) did not increase in the course of these experiments. Thus, the alteration and probably the mechanism of lysosomal enzyme secretion in platelets from pale ear and light ear is different in kidney and platelets. The amount of lysosomal enzymes...
secreted from ruby-eye and maroon platelets was not significantly reduced from normal. However, secretions from maroon and ruby-eye were significantly different from beige (β-glucuronidase) and from pearl and pallid for both secreted lysosomal enzymes. Thus, the seven mutants can be divided into three groups based on the degree of secretion of lysosomal enzymes after thrombin stimulation.

In patients with storage pool deficiency, the abnormality in lysosomal enzyme secretion was seen only at lower concentrations of thrombin. We examined the release of β-glucuronidase in platelets from normal and mutant mice at three different thrombin concentrations (Fig. 4). The altered lysosomal enzyme secretion in the mutants was evident both at 0.25 and 2.5 U/ml. Both pale ear and light ear had higher than normal secretion; beige, pearl, and pallid had lower than normal secretion; and maroon and ruby-eye were nearly normal at these two thrombin concentrations. At 0.04 U/ml thrombin, no significant difference was observed. Interestingly, the secretion deficiency in platelets from patients with SPD was most evident at this concentration. Thus, mouse platelets and human platelets are different in their sensitivity to thrombin and in their display of the abnormality in lysosomal enzyme secretion.

**DISCUSSION**

The bleeding abnormality in the mouse pigment mutants described in this article is associated with reduced platelet dense granule contents and an actual reduction of platelet dense granules. These mutants,
can be assigned with high probability to the pigment gene, rather than to differing background genes.

There are at least seven independent mouse mutations that simultaneously affect the function of at least three subcellular organelles: melanosomes, lysosomes, and platelet dense granules. We can conclude not only that a single gene can regulate the function of at least three subcellular organelles, but also that these three subcellular organelles likely have at least seven different steps in common in their biosynthesis, processing, and/or secretion. This relationship between three subcellular organelles is found also in humans. In Chediak-Higashi patients, who are characteristically hypopigmented and have giant lysosomes in many cell types, decreased thrombin-stimulated lysosomal enzyme secretion has been reported, in addition to SPD. Hermansky-Pudlak patients, in addition to albinolike appearance and SPD, have been shown to have reduced thrombin-stimulated lysosomal enzyme secretion from platelets in at least one report. The three organelles share several structural and functional features. Melanosomes, lysosomes, and platelet dense granules likely share a common golgi subcellular origin in some cell types and are enclosed by a single limiting membrane. Lysosomes and dense granules share an acid interior as the result of a proton pumping mechanism. Melanosomes also may have an acid pH, as acid phosphatase activity has been found in melanosomes. Additionally, these three organelles can extrude their contents into the extracellular space. It is possible that other specialized subcellular organelles are affected by these same mutations. For example another lysosome-like organelle, the mast cell granule, has been reported to be defective in beige mice. However, mast cell degranulation has been found to be normal in beige mice. It is interesting that a given pigment gene can differentially regulate lysosome secretion in different cell types. For example, the presence of the pale ear and light ear genes cause hypersecretion of lysosomal enzymes from thrombin-treated platelets and hyposecretion from kidney.

The primary defective gene product has not yet been identified in either human SPD or in animal models. However, a large number of physiologic and biochemical abnormalities have been determined. All affected mutants have diluted pigmentation caused by different processes. The pearl gene lesion causes dilution of all main types of pigment. In pale ear, light ear, ruby-eye, and pallid mice, abnormally small melanosomes have been detected, whereas melanosomes in ruby-eye were reported to be altered in shape. Hair follicles and eyes of pallid mice contain decreased numbers of mature melanosomes. The light ear mutant is known to accumulate a ceroid-like pigment in kidney, which has been identified as an accumula-

Table 3. Thrombin-Stimulated Secretion of Platelet Dense Granule Contents and Lysosomal Enzymes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N-α-somatostatin</th>
<th>β-Glucuronidase</th>
<th>β-Galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (10)</td>
<td>91.3 ± 0.9</td>
<td>21.0 ± 1.2</td>
<td>22.6 ± 1.9</td>
</tr>
<tr>
<td>Beige (6)</td>
<td>64.7 ± 4.2*</td>
<td>9.56 ± 1.1*</td>
<td>12.4 ± 2.1‡</td>
</tr>
<tr>
<td>Pearl (4)</td>
<td>61.4 ± 1.2*</td>
<td>11.1 ± 0.4*</td>
<td>11.2 ± 0.2†</td>
</tr>
<tr>
<td>Pallid (6)</td>
<td>67.5 ± 3.0*</td>
<td>9.57 ± 0.6*</td>
<td>10.0 ± 0.7*</td>
</tr>
<tr>
<td>Maroon (4)</td>
<td>76.6 ± 0.9*</td>
<td>17.0 ± 0.7</td>
<td>17.8 ± 0.8</td>
</tr>
<tr>
<td>Ruby-eye (4)</td>
<td>72.5 ± 1.4*</td>
<td>17.9 ± 0.6</td>
<td>18.8 ± 0.4</td>
</tr>
<tr>
<td>Light ear (6)</td>
<td>76.5 ± 1.1*</td>
<td>48.4 ± 4.1*</td>
<td>43.1 ± 4.1‡</td>
</tr>
<tr>
<td>Pale ear (4)</td>
<td>77.4 ± 1.3*</td>
<td>45.6 ± 1.2*</td>
<td>48.8 ± 2.7*</td>
</tr>
</tbody>
</table>

Platelets were treated with 1.25 U thrombin for 3 min, as described in Materials and Methods. Percent secreted represents (secreted/total cellular plus secreted) × 100.

Values represent the mean ± SEM of platelet determinations. The number of mice used is in parentheses. These values include background secretion, minus thrombin, which was similar (typically 5%–6%) for normal and mutant platelets. Significance tests are between normal and individual mutant strains.

*p < 0.001.

†p < 0.01.

‡p < 0.05.

**p < 0.05.

Together with the previously characterized beige and pale ear mice, may be useful as experimental animal models for platelet storage pool deficiency. Thus, the classical symptoms for platelet storage pool deficiency can be caused by mutations at seven distinct genetic loci on different chromosomes (Table 1). The animals used in this experiment were either coisogenic (spontaneous mutations arising in strain C57BL/6J) or congenic (mutations arising in other strains and transferred to strain C57BL/6J by repeated backcross matings). The mice are essentially genetically identical to C57BL/6J, except for the chromosomal site of the pigment mutation. Thus, any abnormality observed

Fig. 4. Release of β-glucuronidase in response to varying concentrations of thrombin. Values are averages from 8 normal and 2–4 mutant mice. The procedure is described in Materials and Methods.
tion of glycolipids and may be similar to that reported in macrophages of HPS patients. The pallid mutant has abnormal development of the otoliths of the inner ear that can be corrected by adding manganese to the diet. However, neither the pigment dilution nor the lysosomal enzyme secretion defect (Novak, unpublished) are affected by this treatment. The pear mutant has been used as a model for night blindness. Recently, defective natural killer cells have been reported in beige mice and Chediak-Higashi patients and to a lesser extent in pallid and pale ear mice. However, others have reported that pale ear, pearl, and ruby eye-2J (an allele of maroon) did not influence natural killer cell activity. Recently, natural killer cells from CHS have been reported to have giant granules that are similar to other cell types. By far the most characterized of the pigment mutants is the beige mouse, an animal model for the Chediak-Higashi syndrome. In patients with Chediak-Higashi syndrome or animal models for this disease, abnormalities in membrane fusion, membrane fluidity, microtubule polymerization, tubulin tyrosinolysis, cyclic nucleotide levels, deficiencies in glycolipids, and a deficiency in a neutral protease have been reported. In members of one family with storage pool deficiency, abnormalities in plasma glycoproteins and glycolipids have been reported. Which of these defects, if any, are the primary lesion and are responsible for the platelet defects remains to be established.

Storage pool deficiency in humans is very complex. The symptoms of SPD have recently been subclassified based on the extent of the deficiency in both dense granules and α granules in individual patients. Whether these symptoms represent identical genetic lesions on diverse genetic backgrounds or whether, in fact, these symptoms represent mutations at different loci has not been established. Genetically distinct animal models are worthwhile for the eventual understanding of the analogous human disease. It should be possible to further subclassify the seven independent mutants as appropriate models for the various sub-classes of human SPD. We are beginning efforts to characterize the α granules in the platelets of these mice by radioimmunoassay for mouse α granule proteins.

It is important to distinguish among the various mouse mutants as to their phenotype and molecular mechanism of action if appropriate models for specific human SPD diseases are to be chosen. Beige mice and Chediak-Higashi patients, in addition to hypopigmentation, have characteristically giant lysosomes. Except for a recent report describing giant melanosomes in two cases with SPD, this symptom has not been described in other forms of storage pool deficiency, including the Hermansky-Pudlak syndrome, or in the fawn hooded rat or in the other mouse mutations described in this report. Our finding that thrombin-induced secretion of lysosomal enzymes is depressed in beige mice, as has been reported for Chediak-Higashi patients, is further evidence that the beige mouse is a suitable model for this particular SPD. We have been able to distinguish further among the platelets isolated from these mutants by the differential effect on thrombin-stimulated lysosomal enzyme secretion. Beige, pearl, and pallid platelets are low secretors. However, pale ear and light ear platelets secrete twofold more than normal of their lysosomal enzyme content despite containing normal concentrations of platelet lysosomal enzymes, clearly different from all other pigment mutants studied. Maroon and ruby eye are not significantly inhibited in secretion. There is one report that albino HPS patients have depressed release of lysosomal enzymes after thrombin treatment. Thus, it is possible that pearl and/or pallid may be the most suitable models for HPS. The elevated secretion of lysosomal enzymes from thrombin-stimulated platelets of pale ear and light ear suggests that platelets are the origin of the 2–3-fold increase in concentration of serum lysosomal enzymes in pale ear and light ear (Novak, unpublished). It is unknown if in vivo clotting in response to injury or disease in this mutant results in elevated blood levels of lysosomal enzymes. We do know that both plasma and total blood concentrations of lysosomal enzymes are normal in the pale ear mutant (Novak, unpublished). The light ear and pale ear mutants not only differ from the other mutants in regard to thrombin-stimulated platelet lysosomal enzyme secretion, but also have significantly higher serotonin levels (this report). These results emphasize that the various pigment mutants, despite many similarities in effects on platelet dense granule content, are affecting platelets by distinct mechanisms.

The study of these established mouse mutations on a controlled genetic background should provide useful information on the mechanisms and interrelations involved in SPD and on the interrelationships among several related subcellular organelles.

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