Platelet Function in the Chediak–Higashi Syndrome

By George R. Buchanan and Robert I. Handin

Platelet function studies were performed on two patients with the Chediak–Higashi syndrome, one of whom had a history of easy bruising unrelated to thrombocytopenia. Both patients had prolonged bleeding times, abnormal platelet aggregation, and a defect of platelet storage granules, manifested by reduced platelet ADP, an increased ATP/ADP ratio, increased adenosine nucleotide specific radioactivity after $^3$H-adenine labeling, and decreased platelet uptake of radioactive 5-hydroxytryptamine. These findings confirm preliminary data in animals with the Chediak–Higashi syndrome, provide an explanation for impaired primary hemostasis in these patients, and illustrate another disorder in which platelet storage-pool deficiency occurs.

The Chediak–Higashi Syndrome (CHS) is a rare genetic disorder which occurs in man and in several other animal species, including mice, cattle, and mink. The molecular basis of the disease is unknown, but morphologically abnormal granules are found in melanocytes, white blood cells, fibroblasts, and other cells. Partial albinism results from impaired packaging of melanin into giant melanosome granules. There is abnormal phagocytic function, manifested by reduced chemotaxis and failure of fusion of phagocytic vesicles with the abnormal lysosomes within neutrophils and macrophages. Most affected patients develop an “accelerated phase” in early childhood, which is characterized by pancytopenia, histiocytic organ infiltrates, and early death. However, a few patients never enter the “accelerated phase” and survive to adulthood.

Many human patients and animals with the Chediak–Higashi syndrome have been noted to have a bleeding tendency. Thrombocytopenia accompanies the accelerated phase and probably explains some hemorrhagic episodes in humans. A platelet function defect has been described in Chediak cattle, but detailed platelet function studies have not, to our knowledge, been described in the literature in humans with CHS. Investigation of platelet function in patients with CHS is of interest for several reasons: (1) the clinical observations of bleeding in some affected patients; (2) the preliminary data suggesting a qualitative platelet defect in Chediak animals; (3) the suspicion that this generalized disorder of granules might also involve platelet secretory granules; and (4) findings of a platelet storage-pool deficiency of serotonin and adenosine nucleotides in some genetic albinos not having CHS.

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We therefore studied the platelet function of two patients with CHS, one a young child in the accelerated phase and the other an adult with a clinically milder form of the disease. The presence of a storage-pool deficiency of adenine nucleotides was demonstrated in both.

MATERIALS AND METHODS

Case Material

Case 1 was a 5-yr-old boy with the typical features of CHS in the “accelerated phase.” He had always had easy bruising, although no other bleeding symptoms were present except for those associated with a terminal episode of disseminated intravascular coagulation. He had intermittent pancytopenia, associated with splenomegaly and spiking fevers, but he generally maintained normal or only moderately reduced platelet counts. At 6 yr of age he died of overwhelming sepsis.

Case 2 is a 32-yr-old male diagnosed as having the CHS at age 16 on the basis of the typical morphological abnormalities in his granulocytes, mild albinism, a history of cutaneous furunculosis, and a progressive peripheral neuropathy. Pancytopenia, splenomegaly, or other findings of the “accelerated phase” have never developed. Except for occasional epistaxis, he has no symptoms of a bleeding diathesis and has tolerated several orthopedic surgical procedures without excessive bleeding.

Normal subjects. Hematologically normal laboratory personnel who were taking no medications known to impair platelet function served as controls for the studies described below.

Routine Studies

Bleeding times were performed by the modified Ivy method using a plastic template. Platelet retention in glass bead columns was measured by Salzman’s method. Platelet morphology was examined on Wright-Giemsa-stained peripheral blood smears prepared from venous blood anticoagulated with EDTA. Platelets were counted by phase microscopy. Prothrombin time and activated partial thromboplastin time were measured by standard methods.

Preparation of Plasma

Nine volumes of venous blood were collected and mixed with one volume of 3.8% trisodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation at 100 g for 10 min. Platelet-poor plasma (PPP) was obtained by centrifugation at 1000 g for 10 min. Platelet counts were performed on the PRP by phase microscopy and appropriate dilutions of the PRP were made with autologous PPP. Plastic equipment was used throughout.

Platelet Nucleotides

Platelet adenine nucleotides were measured by the firefly assay as modified by Holmsen et al., using an Aminco photometer (American Instrument Co., Silver Springs, Md.) to record the initial light flash. PRP (0.5 ml) was labeled at 37°C for 60 minutes with 1.25 μCi of 3H-adenine (27.1 Ci/m mole, New England Nuclear Co., Boston, Mass.) and ethanol-EDTA extracts were prepared for measurement of the labeled adenine nucleotide pool. High-voltage electrophoresis (Savant Instruments, Inc., Hicksville, N.Y.) of the labeled platelet nucleotides was performed according to the method of Holmsen and Weiss, with several modifications. Cellulose plates (Uniplate, Analtech, Inc., Newark, Del.) were used instead of paper strips. Ten microliters of each 3H-adenine labeled ethanol-EDTA extract was placed upon the plate along with 90 nmoles each of nonradioactive adenosine diphosphate (ADP) and adenosine triphosphate (ATP), and electrophoresis was performed for 75 min at 25 V/cm in 0.05 M citrate buffer, pH 3.8. The plates were then air dried, and the ADP and ATP were located by selective absorption after illumination with a 260 nm UV source. The cellulose corresponding to the quenched fluorescence in the nucleotide carrier was scraped off the plate into a plastic test tube and shaken for 2 min with 1.0 ml distilled water. The tubes were centrifuged for 10 min at 1000 g and 0.8 ml of each supernatant solution was added to 10 ml of Aquasol (New England Nuclear Co., Boston, Mass.) counting fluid and counted in a liquid scintillation counter (Nuclear-Chicago, Chicago, Ill.).
Quench correction was calculated with an external standard. Ten microliters of each ethanol-EDTA extract was counted separately to determine the total number of counts added to each plate. The total radioactivity with the electrophoretic mobility of ADP and ATP, specific radioactivity (in counts/nmole) of each nucleotide, and recovery of counts added to the plate were calculated for each patient and three normal controls.

Serotonin (5-Hydroxytryptamine) Studies

5-hydroxytryptamine (5-HT) uptake by platelets was measured according to the method of Weiss et al.16 with slight modification. PRP, with platelet counts adjusted to approximately 300,000/cu mm with autologous PPP, was incubated at 37°C with 2 µM 14C-5-HT (2 14C-5-hydroxytryptamine binoxalate, 48.5 mCi/mmmole, New England Nuclear Co., Boston, Mass.) for 180 min in order to detect any leakage of radioactivity from the labeled platelets.17 Platelet-bound radioactivity was measured by collecting platelets on albumin-coated 0.45 µ Millipore filters (Millipore Corp., Bedford, Mass.) under reduced pressure, as well as by following the decrease in radioactivity in the plasma.

Platelet Aggregometry

PRP was adjusted to a platelet count of approximately 300,000/cu mm, and platelet aggregation was measured by the turbidometric method of Born18 using a Chrono-Log aggregometer (Chrono-Log Corp., Broomall, Pa.). The aggregating agents and their final concentrations in PRP were epinephrine (L-epinephrine bitartrate, Sigma Chemical Co., St. Louis, Mo.) 2.5 × 10^{-5} M, ADP (adenosine 5'-diphosphate, Sigma Chemical Co., St. Louis, Mo.) 6 × 10^{-6} M, and collagen suspension (prepared from bovine achilles tendon, Sigma Chemical Co., St. Louis, Mo.) 70-350 µg (70 µg gave optimal aggregation in normal PRP).

RESULTS

Standard Hematologic Studies

Platelet counts performed over a 10-mo period in case 1 were extremely variable, ranging from 1,000 to 470,000/cu mm. Survival of homologous transfused platelets was extremely short on several occasions when the patient was thrombocytopenic. Case 2 had a normal platelet count at all times. Routine coagulation studies were normal in both patients (Table 1).

Platelet Function Studies

All studies in case 1 were performed at times when the platelet count was over 200,000/cu mm and relatively stable.

**Bleeding time and platelet retention.** Platelet retention on glass bead col-

<table>
<thead>
<tr>
<th>Study</th>
<th>Normal Value</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (per cu mm)</td>
<td>150,000–450,000</td>
<td>Extremely variable*</td>
<td>340,000</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>3–9</td>
<td>Over 25</td>
<td>22</td>
</tr>
<tr>
<td>Platelet retention (%)</td>
<td>20–80</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Prothrombin time (sec)</td>
<td>12–14</td>
<td>11.5</td>
<td>12.0</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (sec)</td>
<td>25–35</td>
<td>33.5</td>
<td>34.2</td>
</tr>
</tbody>
</table>

*Platelet count was 233,000 and 301,000/cu mm on the two occasions when platelet function studies were done.
CASE 1
NORMAL
CONTROL

Fig. 1. Representative platelet aggregation tracings from cases 1 and 2 and from a normal control. Concentrations of the aggregating agents used were: epinephrine $2.5 \times 10^{-5} \text{ M}$, ADP $6 \times 10^{-6} \text{ M}$, collagen $70 \mu\text{g}/0.4 \text{ ml PRP}$.

measurements were diminished in case 1 and low normal in case 2. Both patients had template bleeding times over 20 min (Table 1).

Platelet aggregation. Platelet aggregation with epinephrine and collagen was abnormal in both patients (Fig. 1). In case 1, there was complete absence of the secondary wave after epinephrine and markedly impaired aggregation with both small and large amounts of collagen suspension. Although case 2 had a small secondary wave after epinephrine and aggregation with collagen, the changes in light transmittance after addition of each aggregating agent were much less than normal. Aggregation in response to $6 \times 10^{-6} \text{ M}$ ADP, a concentration which induced a single irreversible wave of aggregation in normal PRP, was normal in each case.

Nucleotides

In both cases 1 and 2, there was a marked reduction in platelet ADP and an increase in the ATP/ADP ratio (Table 2). That this deficiency was of the stor-

| Table 2. Results of Platelet Adenine Nucleotide Measurements |
|-------------------|-----|-----|
|                   | ATP | ADP | ATP/ADP |
| Case 1            | 5.50| 0.45| 12.2    |
| Case 2            | 5.80| 0.85| 6.76    |
| Controls (n = 15) | 6.63| 2.73| 2.65    |
| SD                | (± 0.90)| (± 0.97)| (± 0.80) |

Measurements are in μmoles/10^{11} platelets.
PLATELET FUNCTION IN CHS

Table 3. Distribution of Radioactivity in ADP and ATP in Platelets Labeled With $^3$H-Adenine

<table>
<thead>
<tr>
<th>Labeled Ethanol-EDTA Extract (cpm/10µl)</th>
<th>Specific Radioactivity (cpm/mole)</th>
<th>Recovery of Counts Placed on Plate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP ADP ATP/ADP</td>
<td>ATP ADP</td>
</tr>
<tr>
<td>Case 1</td>
<td>2592 416 6.23</td>
<td>$8.4 \times 10^4$ $1.7 \times 10^5$</td>
</tr>
<tr>
<td>Case 2</td>
<td>2000 417 4.80</td>
<td>$1.3 \times 10^4$ $8.8 \times 10^4$</td>
</tr>
<tr>
<td>Control 1</td>
<td>1285 314 4.09</td>
<td>$1.0 \times 10^4$ $3.8 \times 10^4$</td>
</tr>
<tr>
<td>Control 2</td>
<td>1249 319 3.91</td>
<td>$6.2 \times 10^3$ $5.4 \times 10^4$</td>
</tr>
<tr>
<td>Control 3</td>
<td>1126 400 2.82</td>
<td>$6.5 \times 10^3$ $6.5 \times 10^4$</td>
</tr>
</tbody>
</table>

age pool was confirmed by demonstration that, after $^3$H-adenine labeling, the specific radioactivity of ADP was increased, and, in both cases, the amount of radioactive adenine incorporated into ATP compared to that of ADP was approximately the same as in the controls, suggesting that the metabolic pools of these nucleotides were normal (Table 3). Approximately two-thirds to three-quarters of the total number of radioactive counts placed on the cellulose plates were accounted for within the ADP and ATP spots.

5-HT Uptake

There was an impairment in the total uptake of (2-14C) 5-HT by the platelets of both cases 1 and 2, although the initial slope was normal or only slightly reduced (Fig. 2). In addition, there was a loss of the radioactivity as the platelets were incubated beyond 1 hr. Whereas normal platelets lost only 2%-3% of the maximal radioactivity during prolonged incubation, platelets of case 1 and case 2 lost 32% and 25%, respectively. These findings, too, were consistent with a storage-pool defect.

DISCUSSION

The findings in these two patients are most compatible with marked deficiency in the storage pool of platelet adenine nucleotides. Nonmetabolic ADP

![Fig. 2. Uptake of (2-14C) 5-HT by platelets in PRP adjusted to a platelet count of 300,000/cu mm. The normal curve is the mean value for 15 normal controls and the shaded area encompasses ± 1 SD.](image-url)
and ATP, serotonin, and other substances are normally stored within the dense granules of circulating platelets. When these granules fail to package and store their constituents or are anatomically absent, clinically significant impairment of platelet function may occur. "Storage-pool disease" of platelets is an increasingly large and heterogeneous group of disorders which has recently been described in a number of clinical settings. In many instances, storage-pool disease is a dominantly inherited congenital disorder not associated with other abnormalities. However, it has also been found to be associated with several autosomal or X-linked recessive syndromes—i.e., in some patients with albinism, in children with the Wiskott-Aldrich syndrome, and in several patients with the thrombocytopenia absent radii syndrome. Acquired storage-pool defects have been reported in one patient in association with an antiplatelet antibody, in alcoholics, in patients with acute leukemia, and in individuals with renal insufficiency.

Many such patients have clinical and laboratory evidence of impaired primary hemostasis, e.g., easy bruising, a prolonged bleeding time, and impaired platelet aggregation. The laboratory hallmarks of storage-pool disease (SPD) demonstrated in many such patients are diminished platelet ADP, and, to a lesser extent, ATP; increased ATP/ADP ratio; increased specific radioactivity of ADP and ATP in platelets labeled with radioactive adenine nucleotide precursors; diminished uptake by platelets of radioactive 5-HT; and a progressive loss of radioactivity on prolonged incubation of radioactive 5-HT labeled platelets. The platelets of our two patients with the CHS demonstrate these features.

The mechanism leading to storage-pool deficiency is unknown. In some cases it appears to be an acquired disorder, a consequence of antibody-induced platelet damage, with resultant partial release of granular contents. Although direct evidence is lacking in humans, studies on the fawn-hooded rat, an animal with SPD, indicate that SPD typically results from an abnormality of platelet dense granule packaging within the megakaryocyte rather than from release of granular contents from circulating platelets. Examination of SPD platelets by transmission electron microscopy in several instances has revealed actual decreased numbers of dense granules. Our results have confirmed and extended observations made in the past on CHS platelets. In 1962 Page et al. noted decreased whole blood 5-HT in two patients with CHS. Since nearly all of the 5-HT in the blood is carried in the platelet dense granules, a deficiency of these granules was probably present in those patients. In 1967 it was reported that "giant granules" were seen in electron micrographs of the platelets from patients with CHS, but no further details were given. According to Davis and Douglas, however, platelet granules in both humans and animals with CHS were normal.

Mutant strains of several animal species also have a disease closely resembling human CHS. These animals have a bleeding diathesis, and abnormal platelet function has been demonstrated. Cattle with CHS have recently been shown to have decreased platelet 5-HT levels, diminished uptake of radioactive 5-HT, abnormal platelet aggregation, and decreased platelet nucleotide content. In addition, beige mice with CHS have been noted to have prolonged
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bleeding times. Platelet morphology by transmission electron microscopy is normal, however, in CHS mink and in other animals with CHS.

The reduction in the storage pool of nucleotides within CHS platelets suggests that an abnormality of dense granules is present. Such a defect might be expected, in that most other granule-containing blood cells are abnormal in this condition. However, since some genetic albinos having none of the features of CHS also have SPD, it is possible that the storage-pool deficiency of CHS platelets is related in some way to the albinism rather than to the granule abnormalities found in the white blood cells.

Our findings of a platelet defect in the older patient, who never developed the “accelerated phase,” provide additional evidence that such patients have biochemical and morphological defects similar to those of younger children who rapidly progress to a fatal outcome. In addition, these findings represent another well-defined similarity between humans with CHS and the various animal models of the disease. The results of these studies appear to furnish an explanation for the bleeding symptoms noted in case 1 and in other patients with CHS reported in the literature. They support preliminary data obtained in animals with CHS, and they describe another group of patients with platelet dysfunction due to storage-pool deficiency.

ACKNOWLEDGMENT

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