A Platelet Abnormality in the Chediak–Higashi Syndrome of Man

By Jonathan L. Costa, Anthony S. Fauci, and Sheldon M. Wolff

Platelets from two probands homozygous for the Chediak–Higashi syndrome have approximately 10% of the normal number of serotonin-containing dense bodies as visualized electron microscopically in air-dried whole mounts. Since transport of serotonin across the platelet plasma membrane proceeds at a normal rate, and the few dense bodies present appear to store normal amounts of serotonin, the absence of dense bodies may account for the low platelet serotonin values found in these patients.

The Chediak–Higashi Syndrome (CHS) is inherited as an autosomal recessive genetic defect characterized by pigmented dilution (partial oculocutaneous albinism), abnormally large lysosomes in many granule-containing cells, and increased susceptibility to pyogenic infection. A variety of leukocyte functional abnormalities, such as poor intracellular killing of bacteria, defective chemotaxis, and decreased fusion of lysosomes with phagocytic vacuoles, have also been reported to occur in this syndrome. In addition, low platelet serotonin (5HT) and adenine nucleotide values have been described in both human and animal models of this disease.

Using newly developed techniques for quantitating platelet dense-body content, storage packet size, and 5HT uptake, we have examined CHS platelets in more detail than has previously been possible.

MATERIALS AND METHODS

Blood from two patients homozygous for CHS (described in more detail by Blume and Wolff) was collected in a citrate-EDTA solution; platelet-rich plasma was prepared as described previously. Endogenous 5HT was determined as described by Costa et al. Platelet-dense bodies were counted electron microscopically in air-dried mounts. Transport of exogenous \(^{3}H\)-5HT (Amersham-Searle, 500 mCi/m mole) across the platelet plasma membrane was determined in platelets resuspended in a Tris–sodium chloride buffer containing bovine serum albumin and dextrose. Formaldehyde was used to stop uptake after a 10-sec period at 37°C, and the amount of \(^{3}H\)-labeled material associated with the pellet extracellular space was subtracted from the total amount of \(^{3}H\)-5HT present following the 10-sec incubation period. The release of \(^{3}H\)-5HT from platelets induced by thrombin was measured in resuspended platelets as follows: platelet-rich plasma from normals and the CHS patients was incubated for 30 min at 37°C with \(1 \times 10^{-7}\) M \(^{3}H\)-5HT. Platelets were cooled to 4°C, spun into a pellet, and resuspended in the buffer described above. Human thrombin (final concentration, 2 U/ml) was used to induce the release reaction, which was terminated after 60 sec by the addition of formaldehyde. Dense bodies were counted in air-dried whole mounts of platelets before and after thrombin treatment.
Table 1. Parameters of 5HT Storage and Thrombin-induced Release in Normal and CHS Platelets

<table>
<thead>
<tr>
<th></th>
<th>Normals  (Four Subjects)</th>
<th>Patient I</th>
<th>Patient II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecules 5HT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per platelet x 10⁶*</td>
<td>3.465 ± 1.150</td>
<td>1.124 ± 0.138</td>
<td>1.896 ± 0.350</td>
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<tr>
<td>Prelabeling</td>
<td></td>
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<tr>
<td>³H-5HT released</td>
<td></td>
<td></td>
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<tr>
<td>by thrombin (%)</td>
<td>80.3 ± 2.7</td>
<td>27.7 ± 7.1</td>
<td>32.6 ± 7.4</td>
</tr>
<tr>
<td>Dense bodies per</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 platelets</td>
<td>762</td>
<td>48</td>
<td>78</td>
</tr>
<tr>
<td>Platelets with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zero dense bodies (%)</td>
<td>2</td>
<td>72</td>
<td>48</td>
</tr>
<tr>
<td>Dense bodies per</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 platelets</td>
<td>148</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

*Mean ± SD.

RESULTS

Table 1 compares data obtained from normal platelets with that obtained using CHS platelets. In both CHS probands, the amount of 5HT per platelet was reduced to about 40% of that seen in normal platelets, and the number of dense bodies per 100 platelets was strikingly reduced. In addition, a greatly disproportionate number of platelets from CHS patients contained no dense bodies. Following thrombin treatment, CHS platelets released approximately 30% of their ³H-5HT and 80% of their dense bodies. Normal platelets released about 80% of their ³H-5HT and 80% of their dense-body complement.

A CHS platelet whole mount is shown in Fig. 1. Electron micrographs of platelet whole mounts prepared from the blood of normal subjects have been published.

Table 2 presents data on the uptake in 10 sec of 10⁻⁶ M ³H-5HT by normal and CHS platelets. No statistically significant differences were found between the rates at which normal platelets and CHS platelets accumulated ³H-5HT.

DISCUSSION

It is reasonable to assume that the thrombin-releasable 5HT in CHS platelets is sequestered in dense bodies, since under the treatment conditions used here, normal platelets do not appear to release 5HT which is outside their storage vesicles. On the basis of the amounts of endogenous 5HT released per platelet, we can calculate that each dense body in CHS platelets contains on the average 1.0 x 10⁶ molecules of 5HT. Other work has suggested that dense bodies in normal human platelets can store approximately twice as much 5HT as they usually contain in vivo, or on the order of 1.2 x 10⁶ molecules of 5HT per dense body. Each dense body in CHS platelets thus appears to store an amount of 5HT close to the maximal packet size for normal platelets.

Given the amount of 5HT that appears to be contained in each dense body in CHS platelets, we can also estimate that approximately 6 x 10⁶ molecules of 5HT are held in a non-dense-body (cytoplasmic) compartment. This finding is consistent with data indicating that normal platelets can store considerable amounts of 5HT in a cytoplasmic pool.
Data was obtained utilizing four platelet aliquots from each of four normal subjects.

\[ \text{Mean} \pm \text{SD.} \]

**Table 2. Initial Rate of 5HT Uptake by Normal and CHS Platelets**

<table>
<thead>
<tr>
<th></th>
<th>Normals*</th>
<th>Patient I</th>
<th>Patient II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moles of [^3^\text{H}\text{-5HT} \text{ accumulated/platelet/} \times 10^{20} \text{ sec} ]</td>
<td>9.270 ± 1.549</td>
<td>9.456 ± 1.346</td>
<td>9.261 ± 0.948</td>
</tr>
</tbody>
</table>

*Data was obtained utilizing four platelet aliquots from each of four normal subjects.
†Mean ± SD.

**Fig. 1.** Electron micrograph of an air-dried platelet whole mount prepared from a patient homozygous for the Chediak-Higashi syndrome. Although electron-opaque structures resembling \( \alpha \)-granules are present, no dense bodies are seen. \( \times \) 26,400.
Since the apparent presence of 5HT in a cytoplasmic compartment does not alter the rate at which 3H-5HT is moved across the plasma membrane in CHS platelets, it seems likely that extracellular and cytoplasmic material are not in equilibrium. Active transport may thus proceed against a gradient, or 5HT held in platelet cytoplasm may be different in some fashion from that in the extracellular medium (possibly due to binding to some component in the cytoplasm or to a chemical change of the 5HT molecule itself).

In agreement with results obtained by other workers, the data presented here suggest that human CHS platelets resemble those from patients with storage pool disease. We have, however, explored in CHS platelets certain parameters of serotonergic function not previously investigated with other storage-pool-deficient platelets. Since the initial rate of plasma-membrane transport of 5HT and the maximal packet size of the few dense bodies present appear to be within the normal range, the low platelet 5HT values found in CHS patients may be attributable to the absence of storage sites.

REFERENCES

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