An Ultrastructural Morphometric Analysis of Platelet Giant and Fusion Granules

By Claire M. Payne and Lewis Glasser

Our purpose in this study was to establish ultrastructural morphometric criteria that can be used to define pathologic giant and fusion platelet granules and to determine whether patients with neoplastic myeloproliferative disorders (MPD) can be distinguished from other patients. We have morphometrically analyzed 2,391 giant and fusion granule profiles from 46 patients with neoplastic MPD, 127 other diseased control subjects, and 30 normal subjects using a computerized image analyzer. The largest granule profile observed in normal subjects had an area of 0.51 μ² and a perimeter of 3.21 μ. The most irregularly shaped of the large granule profiles photographed from normal subjects had a form factor (FF) value of 0.31. FF values indicate the degree of deviation of a given granule contour from a circle and is expressed by the formula 4πA/P². A pathologic granule profile was then defined as a granule that exceeded any of these limits. Fifty-seven percent of the patients with neoplastic MPD were determined to have abnormal platelet granule profiles using the aforementioned morphometric criteria. It was determined, however, that morphometrically defined giant- and fusion-type granule profiles were nonspecific and were also found in 20% of the other diseased patients with no clinical evidence of an underlying neoplastic MPD. Morphometry has allowed us to define the upper limits of normal for the area and perimeter of individual platelet granules. Morphometrically defined giant fusion granules were determined to be more prevalent in the neoplastic MPD group, but because of their nonspecificity, may only have diagnostic significance for the individual patient in specific clinical settings. The pathogenesis of platelet fusion granules is discussed.

Electron microscopy has made a significant contribution to our understanding of the normal and defective human platelet. Ultrastructural defects involving platelet granules have been well documented and include a deficiency or absence of dense bodies (Hermansky-Pudlak syndrome), storage pool disease or α-granules (gray platelet syndrome). An ultrastructural description of giant granules was first reported in 1960 in Willebrand-Jürgens disease. Fusion granules (giant, irregularly shaped granules) resulting from an apparent fusion of normal-sized α-granules have since been reported to be characteristic of platelets obtained from patients with preleukemia and myelomonocytic leukemia (MML). If platelet fusion granules were specific, the diagnostic import would be similar to Auer rods, which are fusion granules found in neoplastic granulocytes. Platelet giant granules have, however, been reported, on a subjective basis, in normal human platelets, platelets from patients with nonneoplastic hematologic diseases, hairy cell leukemia, and neoplastic myeloproliferative disorders (MPD) in addition to preleukemia and MML.

Electron microscopic examination of normal platelets in our laboratory revealed occasional granule profiles that also appear fused, irregular in shape, and somewhat larger than the majority of other granule profiles. A qualitative assessment of what constitutes an abnormal granule has hitherto been subjective and does not adequately distinguish normal and pathologic granules. In this study, we have used a computerized image analyzer to morphometrically analyze 2,391 giant and fusion granule profiles from 46 patients with neoplastic MPD, 127 other diseased control subjects, and 30 normal subjects. Our purpose was to establish ultrastructural morphometric criteria that can be used to define the pathologic nature of platelet giant and fusion granule profiles and to determine whether patients with neoplastic MPD can be distinguished from other patients.

Materials and Methods

Patient Selection

Normal subjects. Thirty adult volunteers from our faculty/staff who showed no evidence of an underlying hematologic or nonhematologic disorder were selected.

Steroid-treated normal subjects. This group was chosen because it represents a simple stress-related effect that could be used as a baseline for the stress related to diseased states in general. We have shown in a previous study that a single dose of dexamethasone had an effect on platelet ultrastructure. We have examined this same material in the present study to determine whether steroids had any specific effect on platelet granules. Briefly, 12 adult volunteers were given a single dose of oral dexamethasone (4 mg/m² of body surface area). Presteroid and poststeroid (nine to 13 hours after ingestion) blood samples were then analyzed.

Nonneoplastic, diseased control groups. This selection of patients consists of the following clinical groups.

1. Nonneoplastic platelet abnormalities: 40 patients with reactive thrombocytosis, eight patients with thrombocytopenia, four patients with congenital platelet disorders (two patients with the May-Hegglin anomaly, one patient with the Bernard-Soulier syndrome, and one patient with storage pool disease), and two patients with platelet satellitism.

2. Nonneoplastic WBC abnormalities: reactive (five patients with eosinophilia, six patients with a granulocytic leukemoid reaction, two patients with monocytosis, and seven patients with lymphocytosis) and congenital (one patient with each of the following disorders: Chédiak-Higashi syndrome (CHS), Pelger-Hüët anomaly, chronic granulomatous disease of childhood, severe combined immunodeficiency, and ataxia-telangiectasia).

3. Nonneoplastic RBC abnormalities: four patients with secondary polycythemia, one patient with malignancy-associated anemia, one patient with autoimmune hemolytic anemia, one patient with iron deficiency anemia, and one patient with thalassemia major.

Neoplastic lymphoproliferative disorders (LPD) and plasma cell dyscrasias (PCD). This group of patients consisted of five patients with acute lymphoblastic leukemia, nine patients with chronic lymphocytic leukemia, two patients with the Sézary syndrome, one patient in the leukemic phase of lymphoma, two patients with Burkitt's lymphoma, two patients with nodular sclerosing Hodgkin's...
disease, one patient with plasma cell leukemia, and three patients with plasma cell myeloma.

Neoplastic MPD. The following three types of MPD were included: dysmyelopoietic syndrome (DMPS), eight patients; acute nonlymphoblastic leukemias (French-American-British classification M1 to M6), 25 patients; and chronic MPD, nine patients with chronic myelogenous leukemia (CML), five patients with primary thrombocytosis (PT), one patient with megakaryocytic myelosis, three patients with agnogenic myeloid metaplasia with myelofibrosis, and three patients with polycythemia vera.

Preparation of Buffy Coats for Electron Microscopy

Blood samples were collected by venipuncture using EDTA as an anticoagulant. All healthy volunteers and patients were advised of this procedure and its attendant risks in accordance with our institutional guidelines and gave informed consent. A Buffy coat plug was obtained using the centrifugation procedure and in situ fixation procedures originally described by Anderson. The blood was centrifuged in Wintrobe tubes at 2,500 rpm for ten minutes in a Sorvall GLC-1 swinging bucket centrifuge. The plasma was removed and the Buffy coat plug fixed for two hours in 3% glutaraldehyde (buffered with 0.1 mol/L phosphate buffer, pH 7.2). The Buffy coats were removed and sliced perpendicularly to the top surface of the plug before postfixation in 1% osmium tetroxide as prescribed.35 Platelets prepared in this manner instead of first obtaining a platelet-rich plasma (PRP) fraction allowed for the examination of both heavy (near-white cell layer) and light platelets. The Buffy coats were dehydrated in a graded series of ethanols and embedded in Spurr's low-viscosity epoxy resin.36 One-micron sec-embedments of the plug before postfixation in 1% osmium tetroxide were thin-sectioned with a diamond knife on a MT-5000 ultramicrotome and examined under an HU-12 electron microscope (Hitachi, Mountain View, Calif). The sections were mounted on uncoated 200-mesh copper grids, stained with lead citrate and 5% uranyl acetate (made up in 50% methanol), and examined under an HU-12 electron microscope. Each grid square, therefore, contains the entire grid surface. Each grid square varied from one (some normal subjects) to over 40 (diseased groups) depending upon the frequency of abnormal-appearing granule profiles. For this study we concentrated specifically on different platelets from 30 normal subjects, occasional fused giant platelet profiles present. For this study we concentrated specifically on finding giant and fusion granule profiles, which were rare in all of the normal subjects and few in number in most of the control cases. The very narrow and elongated rod-shaped granules frequently seen in normal platelets were not photographed. The negatives were printed on 8 × 10-in sheets of Kodabromide paper and morphometrically analyzed using a MOP-3 computerized image analyzer. The granule membrane was traced in all cases. In oblique sections in which a portion of the membrane became less distinct, the granule contents were outlined and the resultant tracing made continuous with the more distinct adjacent membrane profiles. The perimeter (P), area (A), and form factor (FF) values were then computed for each granule photographed. FF is a formula that is programmed into our MOP-3 image analyzer and gives a measure of contour irregularity. The degree of irregularity of a given profile is expressed by its FF value, which is obtained by inserting the area and perimeter of the contour into the formula: FF = 4πA/P². If A² is substituted for A and 2r is substituted for P, where r is the radius, all terms cancel out and the FF value for a circle becomes 1.0. The degree of contour irregularity increases as the FF value decreases. A granule contour with a FF value of 0.20 would therefore have a shape that is only 20% of a circle.

Statistical Analysis

The mean megagranule group value and SEM for each morphometric parameter was determined by first obtaining the largest perimeter and area and lowest form factor values for all of the usual granule profiles photographed and analyzed from each patient in each clinical group and then averaging these values within each group. The mean group morphometric values were statistically compared using Student's t-test. The difference between two means was considered statistically significant if the P value was less than .05.

RESULTS

Ultrastructure of Normal Platelets

The normal human platelet (Fig 1A) is ultrastructurally complex and contains mitochondria, a dense tubular system, an open canalicular system, dense bodies, α-granules, microfilaments, microtubules, and glycogen. It is generally believed that platelet organelles with eccentric nucleoids are α-granules (Fig 1A). Some granules without nucleoids could also be α-granules in which the plane of the section does not include the nucleoid. α-granule profiles that include the nucleoid in the plane of the section are variable in size and shape. Occasionally, giant α-granule profiles are observed that also appear round in contour (Fig 1B). The electron density of the α-granule matrix substance is appreciably less, however, than that of the dense granule (Fig 1A). During electron microscopic examination of the profiles of approximately 30,000 different platelets from 30 normal subjects, occasional fused giant α-granule profiles (Figs 2A and 2B) and irregularly shaped granule profiles (Fig 2B) were found. Some of these fusion-type granule profiles appeared qualitatively similar to the fusion granule profiles reported in patients with preleukemia and MML.15

Ultrastructure of Platelet Giant and Fusion Granules from Patients with Neoplastic MPD

A typical fusion granule profile commonly found in patients within the neoplastic MPD group is highly irregular in shape, has appreciable electron density, and consists of the contents of numerous α-granules (Fig 3A). The number of α-granules that fuse to form the giant granules is quite variable. In some profiles, as many as 20 individual granules could be identified within one megagranule profile (Fig 3B).
Fig 1. Electron micrographs of platelet profiles obtained from normal subjects (uranyl acetate, lead citrate). (A) The α-granules (α-G) contain eccentric nucleoids (arrow) and appear round to oval in shape with some profiles slightly larger than mitochondria (M). The dense body (DB) is more electron dense than the α-granules. Also shown are glycogen particles (gly), the cross sections of microtubules (mt), and elements of the open canalicular system (OCS) (original magnification x 46,400; current magnification x 34,800). (B) This platelet profile contains a giant granule that is round in contour and contains two eccentric nucleoids. Also shown is a microtubular cross section (arrow) and elements of the open canalicular system (original magnification x 54,800; current magnification x 41,100).

Occasionally, other cytoplasmic organelles such as ribosomes, mitochondria, and glycogen particles become entrapped within the megagranule.

Morphometric Analysis of Platelet Giant and Fusion Granules From Normal Subjects

The largest granule profile observed in all 30 control subjects had an area of 0.51 μ² and a perimeter of 3.21 μ (Fig 2B). The most irregularly shaped of the large granule profiles had an FF value of 0.31. This FF value represents a contour that is only 31% of a circle. A pathologic granule profile was then defined as a granule profile whose area exceeded 0.51 μ², whose perimeter exceeded 3.21 μ, or whose FF value was less than 0.31. The very narrow and elongated rod-shaped granules frequently seen in normal platelets were not included in this study.
Morphometric and Ultrastructural Analysis of Platelet Giant and Fusion Granules in Diseased Control Subjects With No Evidence of an Underlying Neoplastic MPD

Twenty percent of these diseased control subjects had some abnormal platelet granule profiles using the morphometric criteria outlined above (Table I). The number, however, of morphometrically defined abnormal platelet granule profiles was low in these clinical groups. Only 57 granule profiles were determined to be abnormal after image analysis. Overall, abnormal granule profiles were identified in approximately 0.04% of the platelet profiles from these patients.

Morphometrically defined abnormal granule profiles were
Fig 3. Electron micrographs of platelet profiles obtained from patients with neoplastic MPD (uranyl acetate, lead citrate). (A) Platelet profile from a patient with DMPS. The giant fusion granule shown here has a heterogeneous electron-dense content and a highly irregular contour. This abnormal granule had the largest perimeter and area of the entire neoplastic MPD group. Also shown are mitochondria and elements of the open canalicular system (original magnification × 33,800; current magnification × 25,350). (B) Platelet profile from a patient with erythroleukemia. The giant fusion granule shown here contains the outlines of at least 20 distinct granules. Glycogen particles also form part of the contents of this megagranule. Scattered microtubular cross sections (arrows) and elements of the open canalicular system are present (original magnification × 39,900; current magnification × 29,925). Figure 3B is reprinted with permission from Proc EMSA, pp 786–787, 1983 (San Francisco Press, Box 6800, San Francisco).

identifed in at least one patient in each of the following control groups: steroid-treated normal subjects, nonneoplastic platelet disorders, nonneoplastic WBC disorders, nonneoplastic RBC disorders, and neoplastic LPD and PCD. Scatter plots showing the distribution of the most abnormal granule profiles obtained from each patient in each of these control groups are shown in Figs 4 and 5. The most abnormal granule profiles were defined as the granule profile with either the largest perimeter, the largest area, or the lowest form factor.

Typical giant fusion granule profiles that were indistinguishable from those found in the neoplastic MPD groups were prominent in patients with some congenital platelet disorders such as the May-Hegglin anomaly and the Bernard-Soulier syndrome (Fig 6A).

Patients with reactive thrombocytosis as a group could be distinguished from normal subjects on the basis of the platelet granule perimeter ($P < .001$) and area ($P < .001$) (Table 2). Mean form factor values had no discriminatory value ($P > .1$) (Table 2).
Table 1. Incidence of Abnormal Platelet Granules in Control Groups

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>No. Cases Studied</th>
<th>No. Cases With Abnormal Granule Profiles</th>
<th>No. Granule Profiles Morphometrically Analyzed</th>
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</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>30</td>
<td>--</td>
<td>255</td>
</tr>
<tr>
<td>Normal subjects, steroid treated</td>
<td>12</td>
<td>1</td>
<td>152</td>
</tr>
<tr>
<td>Nonneoplastic platelet disorders</td>
<td>54</td>
<td>14</td>
<td>713</td>
</tr>
<tr>
<td>Nonneoplastic WBC disorders</td>
<td>26</td>
<td>2</td>
<td>267</td>
</tr>
<tr>
<td>Nonneoplastic RBC disorders</td>
<td>8</td>
<td>3</td>
<td>118</td>
</tr>
<tr>
<td>Neoplastic LPD and PCD</td>
<td>27</td>
<td>5</td>
<td>328</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>25</td>
<td>1,833</td>
</tr>
</tbody>
</table>

Nineteen percent of the patients with neoplastic LPD/PCD had abnormal granule profiles (Table 1, Figs 4 and 5). The neoplastic LPD/PCD group could be distinguished from normal subjects using all three morphometric parameters (perimeter, $P < .01$; area, $P < .02$; FF, $P < .02$) (Table 2).

Three out of the 4 patients with secondary polycythemia had abnormal granule profiles (Figs 4 and 5). One patient had a megagranule profile (Fig 6B) that was three times the size of the largest granule profile (Fig 2B) obtained from normal subjects.

Although one subject out of 12 in our steroid-treated control group had a single granule profile that was slightly larger ($0.55 \mu^2$) than the largest granule profile ($0.51 \mu^2$) from untreated normal subjects, there was no statistical difference between the steroid-treated group as a whole and normal subjects using all three morphometric parameters.

Morphometric Comparison of Platelet Giant and Fusion Granules Between Patients With Neoplastic MPD and All Other Groups

Fifty-seven percent of the patients with neoplastic MPD were determined to have abnormal platelet granule profiles (Table 3). The total number of morphometrically defined abnormal platelet granule profiles was five times more numerous in the neoplastic MPD group compared to the diseased control group. One hundred four granule profiles were determined to be abnormal after image analysis. Overall, abnormal granule profiles were identified in approximately 0.2% of the platelet profiles examined.

The morphometric parameters that were most helpful in discriminating between patients with neoplastic MPD and all other normal and diseased individuals as a group were perimeter ($P < .001$) and area ($P < .001$) (Table 2). The difference between the mean FF values calculated from the neoplastic MPD and control groups was not statistically significant ($P > .05$) (Table 2). Scatter plots showing the distribution of the most abnormal granule profiles obtained from each patient in the neoplastic MPD groups are shown in Figs 4 and 5.
Platelet granule perimeter \((P < .001)\) and area \((P < .001)\) distinguished the neoplastic MPD group from the individual clinical groups associated with nonneoplastic cellular proliferations such as granulocytic leukemoid reactions, eosinophilia, monocytosis, and the neoplastic LPD/PCD group (Table 2). Mean granule FF values had no discriminatory value \((P > .5)\).

Patients with neoplastic chronic MPD, most of whom are associated with high platelet counts, could be distinguished as a group from the reactive thrombocytosis group on the basis of all three morphometric parameters: perimeter, \(P < .001\); area, \(P < .01\); FF, \(P < .01\) (Table 2). The large granule profiles photographed from the neoplastic chronic MPD group were twice as large as the large granule profiles from the reactive thrombocytosis group and were more irregular in contour (Table 2). When patients with reactive thrombocytosis were compared as a group to patients with PT (a neoplastic disorder) as a group, only granule perimeter
had discriminatory value \((P < .05)\) (Table 2). On an individual basis, a patient could be diagnosed as having PT only if the perimeter of the platelet granules exceeded the highest perimeter value in the reactive thrombocytosis group. No morphometric parameter was useful, however, in distinguishing patients with PT as a group from patients with other types of neoplastic chronic MPD (Table 2).

Patients with acute nonlymphoblastic leukemia as a group could be distinguished from patients with DMPS using the mean granule area only \((P > .05)\) and FF \((P > .2)\) values were not statistically significant. Twenty-five percent of the patients with DMPS had no morphometrically defined megagranule profiles, and 12% of the patients with acute nonlymphoblastic leukemia had megagranule profiles. The DMPS group had the largest mean megagranule profile area (1.00 \(\mu^2\)) of all groups; these megagranule profiles were four times larger than the largest granule profiles from normal subjects (Table 2). The most abnormal granule profile in the study was also obtained from a patient with DMPS (Fig 3A). The perimeter was 9.8 \(\mu\), the area was 2.2 \(\mu^2\), and the shape was only 23% of a circle (FF = 0.23).

### Table 3. Incidence of Abnormal Platelet Granules in Neoplastic MPD

<table>
<thead>
<tr>
<th>Neoplastic MPD</th>
<th>No. Studied</th>
<th>No. Cases With Abnormal Granule Profiles</th>
<th>No. Granule Profiles Morphometrically Analyzed</th>
</tr>
</thead>
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<tr>
<td>Acute nonlymphoblastic leukemia</td>
<td>7</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>AML</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Acute MML</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Acute progranulocytic leukemia</td>
<td>3</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Acute monoblastic leukemia</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Acute basophilic leukemia</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Erythroleukemia</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>DMPS</td>
<td>8</td>
<td>6</td>
<td>162</td>
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<tr>
<td>Chronic MPD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CML</td>
<td>9</td>
<td>3</td>
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</tr>
<tr>
<td>PT</td>
<td>5</td>
<td>5</td>
<td>84</td>
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<tr>
<td>Megakaryocytic myelositis</td>
<td>1</td>
<td>0</td>
<td>10</td>
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<tr>
<td>Agnogenic myeloid metaplasia with myelofibrosis</td>
<td>3</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>3</td>
<td>3</td>
<td>79</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>26</td>
<td>558</td>
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</table>

### DISCUSSION

In this study we attempted to remove the subjectivity in the identification of abnormal platelet granule profiles by using computerized image analysis techniques. Occasional atypical-appearing granule profiles found in normal subjects can be confused, on a subjective basis, with some of the fusion granule profiles that have been described in patients with preleukemia\(^1\)\(^-\)\(^17\),\(^30\) and MML.\(^1\)\(^-\)\(^17\),\(^30\) We have morphometrically analyzed over 2,000 atypical granules from normal subjects, patients with 13 different neoplastic MPD, and other diseased control subjects that included patients with a variety of reactive and congenital WBC, RBC, and platelet disorders. We have determined, after examining the platelets of 30 normal subjects, that one could define an abnormal granule profile as one whose perimeter exceeded 3.21 \(\mu\), whose area exceeded 0.51 \(\mu^2\), or whose shape was less than 31% of a circle. Maldonado\(^4\) has reported that some giant granules found in normal humans can have a diameter of 0.8 \(\mu\). This would correspond to an area of 0.50 \(\mu^2\) (assuming a circular profile) and is very close to our measured value of 0.51 \(\mu^2\).

Although platelet giant fusion granules were initially reported to be possible cell markers for preleukemia and MML,\(^1\)\(^-\)\(^17\) we have shown using image analysis that they are non-disease-specific and can be identified in 20% of the diseased control patients who have no clinical evidence of an underlying neoplastic MPD. Approximately three times as many patients with neoplastic MPD had morphometrically defined abnormal granule profiles compared with other diseased control subjects. The morphometric parameters that had the greatest discriminatory value were perimeter and area. Since the neoplastic MPD group could be distinguished from the neoplastic LPD/PCD group using these parameters, platelet analysis may have some significance in the diagnosis of poorly differentiated leukemias. Distinguishing PT from secondary thrombocytosis can also be a diagnostic problem. We have morphometrically determined that patients with PT as a group could be distinguished from patients with secondary thrombocytosis as a group when the mean group maximum perimeter values are compared. When diagnosing an individual patient, however, the maximum megagranule perimeter for that patient must exceed the maximum individual value in the secondary thrombocytosis group for the platelet proliferation process to be suggestive of a neoplastic process. Megagranule morphometric analysis, therefore, has specificity but may lack sensitivity.
Ultrastructural studies from other laboratories have indicated that on a subjective basis giant-appearing granule profiles could be found in neoplastic MPD other than preleukemia and MML. These included acute myeloblastic leukemia (AML), acute monocytic leukemia (AMoL), CML, PT, and MF. In addition to preleukemia and MML, we have morphometrically confirmed the presence of abnormal giant fusion granules in AML, CML, PT, and MF. We could not morphometrically identify any abnormal granule profiles in our case of AMoL. As a result of this study, we can now add erythroleukemia and polycythemia vera to the list of neoplastic MPD that are known to contain these megagranules. McClure et al described by light microscopy many large platelets with heavy granules in a 5-year-old boy with chronic erythroleukemia. These heavy granules probably correspond to the giant fusion granules that we observed in one of our two cases of erythroleukemia at the ultrastructural level (see Fig 3B).

Platelet giant fusion granules have been previously reported in some congenital WBC disorders. We have morphometrically confirmed the presence of giant fusion granule profiles in one of our two patients with the May-Hegglin anomaly. Giant platelet granules have not been reported in all cases of the CHS. Davis and Douglas, on the other hand, state that the only bone marrow elements that lack giant granules are the megakaryocytes and platelets. Enlarged platelet α-granule profiles were also not identified in the bovine, and mouse homologues of the human Chédiak-Higashi trait. We have not been able to morphometrically identify any large granule profiles in our patient with the CHS.

We have also morphometrically identified for the first time giant fusion granules in the Bernard-Soulier syndrome, which, like the May-Hegglin anomaly, is characterized by the presence of giant platelets. These megagranule profiles were easy to find and were indistinguishable ultrastructurally from the giant fusion granule profiles found in the neoplastic MPD groups. Previous ultrastructural studies on patients with the Bernard-Soulier syndrome did not report the presence of any megagranule profiles. Our case was complicated, however, by the fact that our patient was splenectomized 26 years prior to our ultrastructural evaluation. Although the pitting role that the spleen plays in the removal of Howell-Jolly bodies, autophagic vacuoles, Heinz bodies, and siderotic granules from circulating erythrocytes has been well documented, the role that the spleen plays in the pitting of megagranules from platelets needs to be evaluated.

Among the benign RBC disorders, morphometrically defined megagranule profiles were observed in three of the four patients with secondary polycythemia. The polycythemia in these three patients was secondary to hypoxemia. Two of the patients had chronic obstructive pulmonary disease, and one patient had the pickwickian syndrome. The high incidence of megagranules in this clinical group has not been previously reported.

Although platelet giant fusion granules may be likened to leukocyte Auer rod formation or the pseudo-CHA of leukemia, we have shown in this morphometric study that they are found in many different nonneoplastic conditions. It is therefore possible that the pathogenesis of these megagranules may be similar in both the nonneoplastic and neoplastic conditions. On the other hand, the many different clinical conditions that exist in the diverse patient population studied makes it equally plausible that the formation of giant and fusion granules may have multiple causes.

Our studies suggest that an oxygen-poor microenvironment may be conducive to α-granule fusion with subsequent megagranule formation. Three patients with secondary polycythemia had low PO2 blood levels, and all had platelet giant granules. Support for a hypoxic cause may come from platelet storage experiments. White and Clawson showed that giant granules can occur in normal platelets during prolonged storage. These stored platelets may be exposed to hypoxic conditions. The present study has also shown that morphometrically defined abnormal platelet granule profiles were five times more numerous in the neoplastic MPD group compared to the diseased control group. Thus, additional factors such as abnormal cellular regulatory mechanisms common to the neoplastic state, abnormal granule formation, and changes in the cellular microenvironment may also contribute to megagranule formation.

As a result of this study we have established ultrastructural morphometric criteria that can be used to define the pathologic nature of platelet giant and fusion granule profiles. We have determined, with the use of morphometry, that giant fusion granules are non-disease-specific and can be seen in patients who have no evidence of an underlying neoplastic MPD. Giant fusion granules are, however, more prevalent in the neoplastic MPD group, but because of their nonspecificity, may only have diagnostic significance for the individual patient in specific clinical settings. The formation of megagranules may have multiple pathogenic mechanisms. Factors such as hypoxia, splenectomy, hypersplenism, abnormal cellular regulatory mechanisms, and abnormal thrombopoiesis may play a role in their formation. The mechanism of granule fusion remains to be investigated.

ACKNOWLEDGMENT

The authors are most grateful to Dr Jack M. Layton (Head, Department of Pathology) for his continuous encouragement and support. We also thank Virginia Borduin and Alison Kim for excellent technical assistance and Katie Eckinger for superb typing of the manuscript.

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