Human Megakaryocytes III. Characterization in Myeloproliferative Disorders

By Enrique M. Rabellino, Richard B. Levene, Ralph L. Nachman, and Lawrence L. K. Leung

Abnormal proliferation of the megakaryocytic line was observed in the marrow tissue from patients with myeloproliferative disorders. Megakaryocytes were identified by immunofluorescence using distinct platelet protein markers. Plasma factor VIII antigen (factor VIII:AGN) and platelet glycoproteins IIb and IIIa were detected in normal mature and early megakaryocytes, as well as in a morphologically heterogeneous population of low density marrow cells regarded as atypical megakaryocytes. Atypical megakaryocytes were defined as oval/round 14–35-μm diameter blast-like mononuclear/multinucleated cells bearing platelet protein markers with distinct morphological features, including cytoplasmic vacuolation, variable nuclear/cytoplasmic ratios, and variable cytoplasmic granulation. Atypical megakaryocytes were observed in most chronic myelogenous leukemia (CML) patients and in two patients with polycythemia vera, representing between 60 and 1,840 cells/10⁶ cells (<1.050 g Percoll/cm³). No atypical megakaryocytes were found in (a) 20 normal controls, (b) two patients with essential thrombocytopenia, (c) a patient with thrombocytosis secondary to acute bleeding, and (d) in two patients with CML. Atypical megakaryocytes appear to represent a single-cell population, as demonstrated by a series of double immunofluorescence assays using combinations of five different antiplatelet protein sera. There was a statistically significant correlation between the frequency of atypical megakaryocytes and the presence of immature forms of myeloid cells in blood. Analyses of Fc IgG receptors conducted with two different immunofluorescence systems have demonstrated that phenotypic similarities existed between atypical megakaryocytes and myeloproliferative platelets. Analyses of platelet proteins and differentiation markers on megakaryocytes are useful in elucidating the pathophysiologic alterations occurring in the megakaryocytic compartment in patients with myeloproliferative disorders.

MYELOPROLIFERATIVE disorders consist of a group of diseases characterized by the variable, neoplastic proliferation of the myeloid, erythroid, and megakaryocytic compartments. Chronic myelogenous leukemia (CML), myelofibrosis, polycythemia vera, and essential thrombocytosis represent most readily distinguishable clinical entities. However, changes in the proliferative rate within the different cell compartments frequently results in intermediate clinical conditions and, occasionally, transition of one disorder into another. Dysmegakaryocytosis, leading to production of functionally defective platelets, is frequently present and represents one of the most characteristic features of the syndrome. A number of qualitative defects have been described in myeloproliferative platelets. These defects include: (a) abnormal morphology,® (b) loss of response or increased sensitivity to aggregating agents,® ° (c) decreased number of alpha-adrenergic and prostaglandin D₁ receptors,® ° (d) deficiency in platelet lipoperoxidase activity,® ° (e) acquired platelet storage pool disease,® ° (f) abnormalities in the expression of platelet membrane glycoproteins,® ° and (g) increased expression of Fc receptors.® ° Hemorrhage and thrombosis are frequent complications during the clinical course of these diseases.® ° Although a direct association between the different platelet abnormalities and the bleeding and thrombotic events has not been firmly established, these defects may contribute to the imbalance of hemostasis. Recently, it has been postulated that marrow megakaryocytes may play a role in the pathogenesis of marrow fibrosis observed in myeloproliferative disorders.® °

Studies involving the characterization of megakaryocytes have been greatly facilitated by the development of methods for their isolation in conjunction with the use of selective cell markers.® ° Analysis of acetylcholinesterase activity, a cytochemical marker for murine and rat megakaryocytes, has allowed the identification of various types of early immature marrow megakaryocytes in these systems.® ° Analogously, previous studies have demonstrated that a number of platelet proteins, such as platelet factor 4, factor VIII:AGN, and platelet glycoproteins IIb, IIIa, and IIIa, can be regarded as selective markers for human megakaryocytes.® ° Using these protein markers, early immature megakaryocytes were demonstrated in normal human marrow and in in vitro grown colony megakaryocytes.® ° This article reports the identification of megakaryocytic cells in marrow from patients with myeloproliferative disorders.
ative disorders. Platelet protein markers were detected in a morphologically heterogeneous population of low density marrow cells. These cells were not characteristic of any normal marrow cells and were regarded as atypical megakaryocytes. Comparable phenotypic expression of Fc receptors on atypical megakaryocytes and myeloproliferative platelets was observed. Analyses of platelet proteins and differentiation markers on megakaryocytes are useful in elucidating the pathophysiologic alterations occurring in the megakaryocytic compartment in patients with myeloproliferative disorders.

MATERIALS AND METHODS

Preparation of Cell Suspensions From Human Bone Marrow and Peripheral Blood

Marrow tissue was obtained from aspirates of patients with hematologic disorders and, alternatively, from rib fragments routinely removed from patients undergoing thoracotomy, as previously established by the National Institutes of Health, Bethesda, MD. Expression of Fc receptors on atypical megakaryocytes atypical megakaryocytes. Comparable phenotypic density marrow cells. These cells were not characteristic disorders. Platelet protein markers were detected in a morphologically heterogeneous population of low density marrow cells. These cells were not characteristic of any normal marrow cells and were regarded as atypical megakaryocytes. Comparable phenotypic expression of Fc receptors on atypical megakaryocytes and myeloproliferative platelets was observed. Analyses of platelet proteins and differentiation markers on megakaryocytes are useful in elucidating the pathophysiologic alterations occurring in the megakaryocytic compartment in patients with myeloproliferative disorders.

Preparation of Washed Platelets

Blood was collected in 0.32% sodium citrate in polypropylene tubes. Platelets were prepared by centrifugation of whole citrated blood at 225 g for 10 min at 20°C. The platelet-rich plasma supernatant was then centrifuged at 1,600 g for 20 min at 20°C and subsequently washed 3 times in a 0.12 M sodium chloride solution containing 0.0129 M sodium citrate and 25 mM glucose at pH 6.8. Platelet counts were performed with a Coulter counter (model ZBI; Coulter Electronics Inc., Hialeah, FL).

Immunofluorescence Assays

Monospecific rabbit antisera against purified factor VIII:AGN and isolated glycoproteins Ib and IIIa were prepared and tested for specificity as described.22 Polyspecific antplatelet membrane antigen and an antiplatelet monoclonal antibody were generated and tested as described elsewhere.29 IgG antibody preparations from these antisera were conjugated to either fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC) (both from BBL Microbiological Systems, Becton-Dickinson & Co., Cockeysville, MD), as previously reported.30 For fluorescence staining, smears of the various types of cell preparations were fixed with pure methanol at room temperature for 15 min. After washing with sodium phosphate-buffered saline (PBS), cells were incubated with the different antibody preparations in a wet chamber at 20°C for 30 min. After washing in PBS, cells were examined with a Leitz Ortholux II microscope (E. Leitz, Inc, Rockleigh, NJ) equipped with a Ploem illuminator and phase-contrast optics. Cells were double-assayed for two proteins by staining cell preparations simultaneously or sequentially with a FITC-conjugated antisera and/or an FITC-conjugated specific reagent. The specificity of all immunofluorescence systems used for megakaryocytes/platelets was confirmed by the absolute lack of reactivity of these antisera against any other type of normal marrow and blood cells.

Identification of Early, Mature, and Atypical Megakaryocytes

Marrow cells were examined concomitantly by ultraviolet (UV) and phase-contrast microscopy using a ×100 objective. Mature megakaryocytes were identified by their multiple nuclear lobes, large size, granular cytoplasmic refractive cell membrane, and occasional surface bullae. Early megakaryocytes, on the other hand, were defined as small (10–15 μm) mononuclear lymphoid-like cells bearing platelet markers with smooth cytoplasm and high nuclear/cytoplasmic ratio. Atypical megakaryocytes were defined as round or oval-shaped mononuclear cells with marked cytoplasmic vacuolation of diameters ranging between 14 and 35 μm, bearing at least one of the platelet protein markers studied. Other morphological features included blastlike appearance, different nuclear/cytoplasmic ratios, variable cytoplasmic granulation, and occasional multinucleated configuration. Although most atypical megakaryocytes were mononuclear and a few of size 14–15 μm diameter, cytoplasmic vacuolation and heterogeneous granulation made them distinctly different from lymphoid-like early megakaryocytes. Occasionally, medium-sized atypical megakaryocytes with large oval nucleus and scanty granulation resembled immature myeloid-erythroid precursors (myeloblasts, promyeloblasts, erythroblasts) found in myeloproliferative, but not control, marrow. However, these latter cells did not bear any of the platelet markers tested.

In certain experiments, smears of cell preparations were counterstained with Harris-hematoxylin prior to immunofluorescence assay by incubating methanol-fixed smears with Harris-hematoxylin staining solution at 20°C for 10 min and washing with PBS. The Harris-hematoxylin was prepared with 1 g hematoxylin (MCB Manufacturing Chemists, Gibbstown, NJ), 20 g aluminum ammonium sulfate (Sigma Chemical Co.), and 0.5 g mercuric oxide (Mallinkrodt Inc., St. Louis, MO).31 Additionally, 4% glacial acetic acid was added immediately prior to use.
Detection of Fc IgG Receptors (FcR)

Studies of membrane receptors for the Fc portion of IgG were performed by direct immunofluorescence as previously described. For detection of FcR, suspensions of megakaryocyte-enriched marrow cell preparations and washed platelets were incubated for 30 min with soluble immune complexes prepared at antibody excess. Two types of FITC-conjugated immune complexes were used, one generated with ovalbumin-IgG rabbit antiovalbumin and the other prepared with Keyhole limpet hemocyanin (KLH) IgG rabbit anti-KLH. After staining for FcR, cells were smeared, fixed, and stained with an antiplatelet glycoprotein IIa preparation conjugated to TRITC. Cells were examined under fluorescence microscopy, as mentioned above.

RESULTS

Megakaryocytes in Normal and Myeloproliferative Marrow

Marrow cells from normal individuals and patients with various myeloproliferative disorders were studied for expression of different platelet protein markers by immunofluorescence. Normal marrow tissue contained classical mature and small mononuclear lymphoid-like early megakaryocytes, as previously described (Fig. 1). Differential analysis of marrow cell preparations from patients with myeloproliferative syndromes disclosed that cells bearing platelet markers represented a morphologically heterogeneous population of cells. In addition to mature and early megakaryocytes, marrow from most patients with myeloproliferative syndromes contained a proportion of nucleated cells bearing platelet markers not readily recognizable by their morphology as mature or early megakaryocytes. These cells, regarded as megakaryocytic in nature, were referred to as atypical megakaryocytes. Atypical megakaryocytes (Fig. 2) were identified on the basis of the expression of at least one of the megakaryocyte/platelet markers studied in conjunction with a number of morphological features not observed within normal megakaryocytes. These features included: (a) cytoplasmic vacuolation, (b) size ranging between 14 and 35 μm in diameter, (c) differing degree and heterogeneous distribution of cytoplasmic granulation, and (d) variable nuclear/cytoplasmic ratios. Although the majority of atypical megakaryocytes contained a simple round or oval nucleus, occasionally cells that appeared to be polynucleated were observed. The use of platelet protein markers was essential for the recognition of atypical megakaryocytes because of their dissimilarities with mature and early megakaryocytes and because a few atypical megakaryocytes (1%–2%) in certain CML patients resembled blast cells of presumably erythroid or myeloid origin that were not seen in marrow controls.

Cells bearing platelet glycoprotein IIb represented approximately 11/10^6 unseparated nucleated cells in

Fig. 1. Isolated low density normal marrow cells were methanol-fixed and stained by immunofluorescence for platelet glycoprotein IIb. Cells were examined under u.v. and phase-contrast microscopy (×1,200). (a–a') A classical mature megakaryocyte of about 35 μm diameter with pollylobulated nucleus and granular cytoplasm. (b–b') An early lymphoid-like mononuclear megakaryocyte of approximately 12 μm diameter and high nuclear/cytoplasmic ratio.

Fig. 2. Isolated low density marrow cells from patients with myeloproliferative disorders were methanol-fixed and stained by immunofluorescence for platelet glycoproteins IIb (FITC, yellow) or IIa (TRITC, red) and examined concomitantly under u.v. and phase-contrast microscopy (×1,200). (a–a' and b–b') Examples of atypical megakaryocytes with extensive vacuolation and diameters of 21 and 14 μm, respectively. (c–c') A vacuolated cell with round nucleus is accompanied by a medium-sized pollylobulated cell of approximately 25 μm in diameter.
normal controls and 1710^4 unseparated nucleated cells in patients with CML and other myeloproliferative disorders (Table 1). After density centrifugation, over 98% of marrow cells bearing platelet glycoprotein IIb were found within the gradient fraction of density <1.050 g Percoll/cu cm. In patients with myeloproliferative syndromes, low density (<1.050 g Percoll/cu cm) cells containing platelet glycoprotein IIb represented approximately 1,210/10^4 nucleated cells, while in controls, positively stained cells comprised only 193/10^4 cells.

In addition to platelet glycoprotein IIb, other selective markers for human megakaryocytes, such as factor VIII:AGN and platelet glycoprotein IIIa, were investigated (Table 2). Marrow cells bearing platelet glycoproteins IIIa and factor VIII:AGN were found at frequencies comparable with marrow cells bearing platelet glycoprotein IIb. Distribution analysis of nucleated marrow cells reacting with a platelet polyspecific antiserum and an antiplatelet monoclonal antibody was equivalent to that of the platelet protein markers. Moreover, using a combined fluorescent assay for two or more different markers, such as platelet glycoprotein IIb/factor VIII:AGN and platelet glycoprotein IIIa/platelet polyspecific antiserum, it was demonstrated that marrow cells bearing the various platelet markers tested overlapped, suggesting that they comprised the same cell population.

The relative frequency of megakaryocytic cells as detected by platelet protein markers in low density marrow cells from patients with myeloproliferative disorders and controls are indicated in Table 3. Atypical megakaryocytes were detected in 7 of the 9 CML patients and in the 2 polycythemia patients studied, representing between 60 and 1,840 cells/10^4 low density cells (<1.050 g Percoll/cu cm). No atypical megakaryocytes were detected in marrow tissue from two patients with CML (patients 10 and 11, Table 3), two patients with essential thrombocythemia (patients 12 and 13, Table 3), one patient with aplastic anemia, and one patient with postbleeding thrombocytosis, as well as 20 normal controls. The two CML patients without atypical marrow megakaryocytes did not differ clinically from the other seven CML patients in whom atypical megakaryocytes were found in the bone marrow. Atypical megakaryocytes were associated with either an increased or normal number of mature and early megakaryocytes, which ranged from 20 to 2,480 and from 20 to 600/10^4 low density cells, respectively.

**Correlation of Megakaryocyte Type With Platelet Counts and Circulating Immature Myeloid Cells**

In an attempt to further characterize megakaryocytes in myeloproliferative disorders, the frequency of the various types of megakaryocytes was correlated with the platelet counts. A scatter plot analysis of the square root of the frequency of megakaryocytes and platelets was performed. In normals, an appreciable linear correlation was found between megakaryocytes and platelets (r = 0.66; p < 0.05). By contrast, in patients with myeloproliferative syndromes, no statistically significant correlation was observed between either atypical or all types of megakaryocytes and platelet counts (r = 0.06; p > 0.05; and r = 0.14, p > 0.05, respectively). In addition, a statistically significant correlation was observed between atypical megakaryocytes and the presence of immature myeloid cells in peripheral blood. From a series of regression analyses, the line of best fit was determined to be an exponential relationship (Ln y = Ln 6.22 + 1.03x) with r = 0.96 and p < 0.01 (Fig. 3). In parallel studies of low density peripheral blood cells, atypical megakaryocytes were demonstrated only in three patients (patients 3, 4, and 7, Table 3), which corresponds to those that had the

Table 1: Identification of Bone Marrow Cells Bearing Platelet Glycoprotein IIb by Immunofluorescence

<table>
<thead>
<tr>
<th>Marrow Cells</th>
<th>Patients With Myeloproliferative Syndromes</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unseparated</td>
<td>17 (6-25)*</td>
<td>11 (4-16)</td>
</tr>
<tr>
<td>After density centrifugation</td>
<td>1,210 (130-4,360)</td>
<td>193 (73-313)</td>
</tr>
<tr>
<td>&lt;1.050 g Percoll/cu cm</td>
<td>&lt;0.1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;=1.050 g Percoll/cu cm</td>
<td>1,130</td>
<td>70</td>
</tr>
<tr>
<td>Factor VIII:AGN</td>
<td>1,150</td>
<td>96</td>
</tr>
<tr>
<td>Platelet glycoprotein IIb</td>
<td>1,220</td>
<td>74</td>
</tr>
<tr>
<td>Platelet glycoprotein IIIa</td>
<td>1,280</td>
<td>58</td>
</tr>
<tr>
<td>Platelet glycoprotein IIb + factor VIII:AGN</td>
<td>1,140</td>
<td>76</td>
</tr>
<tr>
<td>Platelet glycoprotein IIIa + platelet polyspecific antimembrane</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-bovine serum albumin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-KLH</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean values of an experiment. Incidence of stained cells was established by counting at least 5 x 10^6 cells in each preparation.

Table 2: Identification of Bone Marrow Cells Bearing Various Platelet Proteins by Immunofluorescence

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Stained Nucleated Cells/10^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Density (&lt;1.050 g Percoll/cu cm)</td>
<td>Marrow Cells</td>
</tr>
<tr>
<td>CML Patients</td>
<td>Control Subjects</td>
</tr>
<tr>
<td>Platelet glycoprotein IIb</td>
<td>1,130*</td>
</tr>
<tr>
<td>Platelet glycoprotein IIIa</td>
<td>1,150</td>
</tr>
<tr>
<td>Factor VIII:AGN</td>
<td>1,220</td>
</tr>
<tr>
<td>Platelet polyspecific antimembrane</td>
<td>1,280</td>
</tr>
<tr>
<td>Antiplatelet monoclonal antibody</td>
<td>1,150</td>
</tr>
<tr>
<td>Platelet glycoprotein IIb + factor VIII:AGN</td>
<td>1,300</td>
</tr>
<tr>
<td>Platelet glycoprotein IIIa + platelet polyspecific antimembrane</td>
<td>1,140</td>
</tr>
<tr>
<td>Anti-bovine serum albumin</td>
<td>0</td>
</tr>
<tr>
<td>Anti-KLH</td>
<td>0</td>
</tr>
</tbody>
</table>

*Platelet glycoprotein IIIa-bearing cells are relatively higher than that usually found in normal controls.
highest frequency of circulating immature myeloid cells. Atypical megakaryocytes in peripheral blood represented 80, 600, and 70 per $10^4$ low density nucleated cells in patients 3, 4, and 7 respectively.

### Table 3. Differential Analysis of Marrow Cells Bearing Platelet Glycoprotein IIb

<table>
<thead>
<tr>
<th>Patients</th>
<th>Diagnosis</th>
<th>Atypical Megakaryocytes</th>
<th>Early Megakaryocytes</th>
<th>Mature Megakaryocytes</th>
<th>Platelets ($\times 10^5$/Liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CML†</td>
<td>1,840</td>
<td>240</td>
<td>2,280</td>
<td>403</td>
</tr>
<tr>
<td>2</td>
<td>CML</td>
<td>960</td>
<td>260</td>
<td>440</td>
<td>674</td>
</tr>
<tr>
<td>3</td>
<td>CML</td>
<td>770</td>
<td>600</td>
<td>2,480</td>
<td>802</td>
</tr>
<tr>
<td>4</td>
<td>CML</td>
<td>600</td>
<td>290</td>
<td>270</td>
<td>674</td>
</tr>
<tr>
<td>5</td>
<td>P. vera</td>
<td>150</td>
<td>160</td>
<td>89</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>P. vera</td>
<td>98</td>
<td>105</td>
<td>182</td>
<td>550</td>
</tr>
<tr>
<td>7</td>
<td>CML</td>
<td>90</td>
<td>20</td>
<td>20</td>
<td>339</td>
</tr>
<tr>
<td>8</td>
<td>CML</td>
<td>64</td>
<td>20</td>
<td>85</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>CML</td>
<td>60</td>
<td>50</td>
<td>50</td>
<td>710</td>
</tr>
<tr>
<td>10</td>
<td>CML</td>
<td>0</td>
<td>40</td>
<td>200</td>
<td>537</td>
</tr>
<tr>
<td>11</td>
<td>CML</td>
<td>0</td>
<td>20</td>
<td>250</td>
<td>184</td>
</tr>
<tr>
<td>12</td>
<td>Ess. thr.</td>
<td>0</td>
<td>23</td>
<td>4,190</td>
<td>850</td>
</tr>
<tr>
<td>13</td>
<td>Ess. thr.</td>
<td>0</td>
<td>50</td>
<td>1,430</td>
<td>1,000</td>
</tr>
<tr>
<td>14</td>
<td>Postbleed thromb.</td>
<td>0</td>
<td>90</td>
<td>180</td>
<td>976</td>
</tr>
<tr>
<td>15</td>
<td>Apl. ane.</td>
<td>0</td>
<td>5</td>
<td>30</td>
<td>150</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0</td>
<td>40 ± 14$\dagger$</td>
<td>150 ± 114$\dagger$</td>
<td>306 ± 27</td>
</tr>
</tbody>
</table>

*Frequency of cells bearing platelet glycoprotein IIb was established by counting at least $5 \times 10^4$ cells in each preparation. Mature megakaryocytes were identified by their large size, multilobulated nucleus, granular cytoplasm, and surface bullae. Early megakaryocytes were identified as small mononuclear cells, lymphoid-like, and high nuclear/cytoplasm ratio. Atypical megakaryocytes were defined as mononuclear cells bearing platelet glycoprotein IIb, containing cytoplasmic vacuolation, size ranging from 1.4 to 35 μm, and variable cytoplasmic granulation and differing nuclear/cytoplasmic ratios.†CML, chronic myelogenous leukemia; Ess. thr., essential thrombocythemia; P. vera, polycythemia vera; Postbleed thromb., postbleeding thrombocytosis; Apl. ane., aplastic anemia.§Mean values ± 1 SD.

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### Megakaryocyte and Platelet Fc Receptors

Megakaryocytes from patients with myeloproliferative disorders give rise to platelets with various abnormalities, including the increased expression of Fc receptors. Hence, studies were undertaken to analyze the expression of Fc receptors in myeloproliferative megakaryocytes. Fc receptors in megakaryocytes and platelets were investigated, in parallel, using the FITC KLH-anti-KLH and FITC ovalbumin-antiovalbumin systems. The frequency of Fc receptors in marrow megakaryocytes was established by counterstaining smeared cells for platelet glycoprotein IIa with a TRITC-conjugated antiserum following membrane staining for membrane Fc receptors. The various types of marrow megakaryocytes (early, mature, and atypical) were established by concomitant examination of stained cells under phase-contrast microscopy. As shown in Table 4, the vast majority of normal and CML megakaryocytes and platelets contained detectable ovalbumin–anti-ovalbumin receptors. More importantly, almost all atypical CML megakaryocytes and platelets contained easily detectable ovalbumin–anti-ovalbumin Fc receptors, suggesting that myeloproliferative platelets had phenotypic similarities with atypical megakaryocytes. It was of interest that, in CML patients, approxi-
ultimately one-third of the early and mature megakaryocytes had ovalbumin-anti ovalbumin FcR.

**DISCUSSION**

Abnormal proliferation of the megakaryocytic line was demonstrated in the marrow tissue from patients with myeloproliferative disorders. Megakaryocytic cells were identified by immunofluorescence using monospecific antisera against factor VIII:AGN, platelet glycoproteins IIb and IIia, as well as a polyclonal antiplatelet membrane antiserum. These proteins have been previously established as selective markers for human marrow megakaryocytes. Proliferation of the megakaryocytic line has been reported in the literature as a feature of various disorders, including acute micromegakaryoblastic leukemia, megakaryocytic myelosis, and aleukemic megakaryocytic leukemia. Such an expansion of the megakaryocytic compartment has been characterized by cells with a number of different histologic features including small mononuclear cells (micromegakaryocytes), blasts, and intermediate forms with various morphological aberrations.

Previously, recognition of megakaryocytic proliferation has primarily been based on the identification of certain characteristic cytoplasmic structures, such as the demarcation membrane system, alpha granules, and platelet peroxidase activity. These studies at the electron microscopic level are not suitable for application to routine diagnostic hematology. More recently, Levine et al. have reported that cell size, in conjunction with nuclear configuration, may be a reliable criterion to identify marrow megakaryocytes. In normal human marrow cell suspension, most megakaryocytes have a cell diameter of \( \geq 14 \mu m \). Analogous studies in smears did show that the threshold value for megakaryocytes was approximately \( 19-23 \mu m \). Although a threshold value of cell diameter facilitates identification of megakaryocytes from other cell types, it excludes a significant proportion of small megakaryocytes. Moreover, this criterion may not apply to marrow cells from patients with disorders with hyperplasia of the myeloid-erythroid elements with increased percentage of large immature forms (\( \geq 14 \mu m \) diameters). Thus, recognition of megakaryocyte blasts and precursors by conventional microscopy has remained very difficult, if not impossible, and has been mainly based on subjective morphological criteria. The techniques described in this study to analyze marrow megakaryocytes are highly specific, easy to perform, and permit a quantitative assessment of the megakaryocytic cell compartments. Atypical megakaryocytes represented a group of cells with an array of morphological features uncharacteristic of any normal blood or marrow cells and, thus far, in the single-cell population, as indicated by a series of double-immunofluorescence assays using combinations of five different antiplatelet protein sera. The megakaryocytic nature of atypical megakaryocytes was confirmed by the expression of various cell markers distinct for the megakaryocytic-platelet lineage. Morphological abnormalities have been described for megakaryocytes from chronic granulocytic leukemia by electron microscopy. Saarni and Linman have described the presence of “atypical” multinucleated megakaryocytes with bizarre forms in myelomonocytic leukemia. Brecher has described atypical forms of megakaryocytes in a series of seven patients with acute and chronic myeloid leukemia, as well as in patients with myeloma and two refractory anemias. The presence of atypical megakaryocytes was regarded as reflecting profound hematopoietic disturbance affecting the early blood cell precursors. The origin, pathophysiologic and clinical significance of these cells, however, remain to be established. Two possibilities are hypothesized:

1. Atypical megakaryocytes may be associated with the neoplastic clone, as suggested by the statistically significant correlation between the frequency of atypical megakaryocytes and the presence in blood of immature forms of myeloid cells.
2. Atypical megakaryocytes represent a population of dedifferentiated megakaryocytes, arising from the normal progenitors.

Future studies on clonal chromosomal changes and restricted expression of glucose-6-phosphate dehydrogenase isoenzymes will provide more definitive infor-
mation concerning the origin of atypical megakaryocytes.

Analyses of Fc receptors were conducted with two different immunofluorescence systems. Using the KLH-anti-KLH system, Fc receptors were detected in virtually all megakaryocytes and platelets tested. Alternatively, Fc receptors for the ovalbumin-antiovalbumin system were expressed only in a minor proportion of normal megakaryocytes and platelets. By contrast, virtually all atypical megakaryocytes and myeloproliferative platelets bore ovalbumin-antiovalbumin Fc receptors. Thus, a phenotypic similarity was demonstrated between atypical megakaryocytes and platelets, suggesting that myeloproliferative platelets may have been derived from the neoplastic clone.

Heterogeneity has been recognized within membrane Fc receptors of macrophages and lymphocytes. The detection of various Fc receptors with restricted specificity has been related to the nature of the immunocomplexes, including molecular weight of the antigen, size of immune complexes, and isotype of the immunoglobulin G molecule. In our studies, the differences in reactivity of the FITC-conjugated immune complexes with both myeloproliferative and normal megakaryocytes and platelets appear to be due to the presence of two distinct Fc receptors. Previous studies have demonstrated that the KLH-anti-KLH complexes interact with an Fc receptor related to the platelet membrane glycoprotein Ib complex, which mediates platelet agglutination by factor VIII:AGN and ristocetin. The ovalbumin-antiovalbumin complexes, on the other hand, interact with an Fc receptor that is not related to the glycoprotein Ib system. The expression of the ovalbumin-antiovalbumin Fc receptors in atypical megakaryocytes and myeloproliferative platelets may reflect developmental irregularities in the cell membrane.

The results from these studies demonstrate that the frequency of megakaryocytic cells in this group of patients was higher than had been reported previously. It is anticipated that analysis of megakaryocytic cells using specific protein and differentiation markers will provide new insights into the origin and the extent of megakaryocyte proliferation. Moreover, it will facilitate the diagnosis and enable a more accurate classification of the myeloproliferative disorders.

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