Growth Characteristics of Circulating Hematopoietic Progenitor Cells From Patients With Essential Thrombocytemia

By Eric M. Mazur, Janet Lee Cohen, and Lee Bogart

Peripheral blood mononuclear cells from five patients with essential thrombocytemia (ET) were cultured in vitro to evaluate restricted megakaryocytic (CFU-Meg), myeloid (CFU-GM), and erythroid (BFU-E) progenitor cell development. Varying concentrations of aplastic canine serum served as the source of megakaryocyte colony-stimulating activity, and cultured megakaryocyte ploidy distributions were determined by Feulgen staining and microfluorometry. Megakaryocyte colony growth was strikingly abnormal in all five patients evaluated. Four of the 5 had a marked expansion in the concentration of circulating CFU-Meg and 3 of 4 manifested abnormalities in cultured megakaryocyte colony size (2 unusually large and 1 small). Unstimulated megakaryocyte colony growth was substantially increased in three patients. However, the fraction of megakaryocyte progenitors in cell cycle was near or below normal in all instances. Endomitotic megakaryocyte development was disordered in each of the four ET patients in whom it was evaluable. In normal subjects, mean megakaryocyte ploidy values vary bisphonally with aplastic canine serum concentration and peak at 13.2 N following 12 to 15 days of culture. In contrast, day 12 mean ploidy values in cultures from the ET patients remained low at all aplastic canine serum concentrations and reached a maximum averaging only 8.4 N. Three patients were evaluated serially at extended culture durations of up to 21 days. The cultured megakaryocyte ploidy was unchanged during this interval for two of the patients. For the third patient, ploidy increased steadily, reaching abnormally high ploidy values by day 21. Progenitor cell expansion was limited to the megakaryocyte line in three patients. However, two patients had substantial increases in CFU-GM, one of whom also had a marked increase in BFU-E. There was no significant unstimulated colony growth by either CFU-GM or BFU-E. These data indicate that ET is usually characterized by an expansion in the concentration of circulating CFU-Meg in vivo which manifest both disordered replication and endoreduplication in vitro.

ESSENTIAL thrombocytemia (ET) is a clinical disorder of excess platelet production that is traditionally considered to be one of the myeloproliferative syndromes as defined by Dameshek in 1951. In common with the other myeloproliferative syndromes, ET is a clonal disease originating at the level of the multipotent hematopoietic stem cell. The reasons for its primary clinical expression in the megakaryocyte-platelet line have not been clearly defined. Potential explanations include: (a) an inappropriate expansion in the number of restricted or high megakaryocyte potential progenitor cells resulting from increased input from the pluripotent hematopoietic stem cell pool, (b) an expansion of megakaryocyte progenitors due to accelerated self-renewal at the committed stem cell level, (c) excessive mitotic and/or endomitotic megakaryocyte development in the presence of a normal regulatory apparatus, and (d) autonomous production of megakaryocytopoietic growth factors.

The development of techniques permitting in vitro growth of human megakaryocytic progenitors (CFU-Megs) and identification of serum megakaryocyte colony-stimulating activity (Meg-CSA) have created new opportunities for studying the pathophysiology of ET. Our laboratory recently defined the relationship between varying concentrations of serum Meg-CSA and the characteristics of normal human megakaryocyte colony development in vitro. Knowledge of this normal relationship provided the framework in which the growth of CFU-Megs from five patients with ET was evaluated in this investigation.

MATERIALS AND METHODS

Human subjects. Peripheral blood for progenitor cell cultures and normal AB sera were obtained by routine venipuncture from consenting healthy adult donors and from five patients with ET, all of whom provided written informed consent. Diagnostic criteria for essential thrombocytemia were adapted from those established by the Polycythemia Vera Study Group. All patients had sustained platelet counts of >600,000/μL with no known underlying cause of reactive thrombocytosis. Hemoglobin was normal or low in all instances. Either stable or iron therapy. None of the patients was iron deficient by serologic criteria. Fibrosis was absent or involved less than one-third of the bone marrow biopsy area, and a leukoerythroblastic peripheral blood picture was not present. Cytogenetics were only obtained in patient 2, and the Philadelphia chromosome was not demonstrated (Table 1).

Canine sera. Aplastic canine sera were prepared as previously described. The aplastic canine sera used in this investigation were harvested from two animals whose platelet counts were 4,000/μL and 8,000/μL when they were killed, and both lots were comparable in their megakaryocyte colony stimulating activity.

Plasma clot megakaryocyte cultures. Peripheral blood from patients and normal volunteers was diluted 1:1 with α-medium minus nucleosides (GIBCO Laboratories, Grand Island, NY) containing preservative-free heparin at 10 U/mL. Mononuclear cells were isolated by Ficoll 400-sodium diatrizoate (Pharmacia, Piscataway, NJ) density centrifugation. The cells were suspended in α-medium containing 20% AB serum, and plastic adherent cells were removed by a 1-hour incubation at 37°C in 100-mm polystyrene...
Table 1. Clinical Characteristics of Patients With Essential Thrombocythemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Platelet Count x 10^3</th>
<th>Mean Platelet Volume (fl)</th>
<th>Hemoglobin (g/dL)</th>
<th>WBC Count/μL</th>
<th>Bone Marrow Morphology</th>
<th>Treatment Prior to Evaluation</th>
<th>Clinical Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>F</td>
<td>952,000</td>
<td>7.5</td>
<td>13.6</td>
<td>7,400</td>
<td>ND (refused)</td>
<td>None</td>
<td>Chronic gastrointestinal bleeding at presentation; no further complications without therapy for 3½ years</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>F</td>
<td>1,658,000</td>
<td>8.2</td>
<td>13.5</td>
<td>15,500</td>
<td>Megakaryocytic hyperplasia with both hyperlobulation and hypolobation of nuclei</td>
<td>None</td>
<td>Treated with busulfan for rising platelet count; no complications</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>M</td>
<td>1,072,000</td>
<td>ND</td>
<td>11.6</td>
<td>9,500</td>
<td>Minimal focal fibrosis</td>
<td>None</td>
<td>Chronic GI bleeding and anagia pectoris; improved after control of the platelet count with busulfan</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>M</td>
<td>720,000</td>
<td>6.6</td>
<td>13.2</td>
<td>5,100</td>
<td>Focal fibrosis with moderate megakaryocytic hyperplasia</td>
<td>None</td>
<td>Stable without therapy for 3 years; no complications</td>
</tr>
<tr>
<td>5</td>
<td>84</td>
<td>F</td>
<td>864,000</td>
<td>ND</td>
<td>11.5</td>
<td>20,400</td>
<td>Early fibrosis with marked megakaryocytic hyperplasia and mild dysplasia</td>
<td>Busulfan 5 and 1 years previously</td>
<td>Spontaneous soft tissue hemorrhage 5 years previously associated with platelet count of 2 million; stable since on intermittent busulfan.</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not done; GI, gastrointestinal.

Aplastic canine serum was added to the cultures at time 0 in concentrations ranging from 0% to 20% (vol/vol), replacing equal volumes of normal AB sera. Megakaryocyte cultures were incubated for 12 days (unless otherwise indicated) at 37°C in a 100% humidified atmosphere of 5% CO2 in air, fixed, and stained immunofluorescently using rabbit antiserum to platelet glycoproteins.5-7,12 One set of the duplicate cultures was stained using a fluorescein-labeled antibody (for enumeration of megakaryocyte colony numbers and colony size); the other set was stained with a rhodamine-labeled antibody (for enumeration of colony numbers and determinations of cultured megakaryocyte ploidy distributions).10,12 Colony counts were comparable between the fluorescein-stained and rhodamine-stained cultures, and the mean of these duplicate values was determined in each experiment.

A megakaryocyte colony was defined as a cluster of three or more intensely fluorescent cells. For colony size determinations, the numbers of cells in each of the colonies within entire culture dishes were quantitated and the arithmetic means were calculated. A maximum of 250 colonies was enumerated in a single 35-mm culture dish, and colony size was determined only below this threshold since at higher colony counts some confluence of colonies was observed.

DNA quantitation. Staining of the colony megakaryocytes for DNA quantitation was performed using the Feulgen technique of Ruch13 modified as we previously reported.10,12 Approximately 100 megakaryocytes were randomly selected in determining the ploidy distribution histograms at each experimental point.10 Readings of diploid cell DNA fluorescence were obtained throughout the culture specimen in a ratio of diploid cells to megakaryocytes of ~1:2, using isolated macrophages and lymphocytes as the diploid reference cells.10,12

When the megakaryocytes in a culture plate were scored, the relative DNA content in each cell was determined using the mean diploid fluorescent signal for that culture plate as the 2 N standard.10,12 Megakaryocytes were assigned to the ploidy level of the nearest modal value of DNA content based on a log-normal distribution of ploidy values, and average ploidy was calculated as the geometric mean.10

Erythroid and myeloid progenitor cell cultures. Erythroid burst-forming progenitors (BFU-E) were assayed in 1-mL plasma clot cultures containing 30% (vol/vol) AB serum.9 BFU-E were maximally stimulated using Step III sheep urinary erythropoietin (Connaught Laboratories, Willowdale, Ontario, Canada) at 4 U/mL and were enumerated after 14 days of culture. To facilitate counting, the plasma-clot BFU-E cultures were fixed in situ with glutaraldehyde and stained with benzidine and hematoxylin. BFU-E-derived colonies were assayed in duplicate and defined using standard criteria.14

Granulocyte-macrophage colony-forming progenitor cells (CFU-GM) were grown in single-layer 0.3% agar cultures prepared as we described.9 Colony-stimulating activity was provided by 10% (vol/vol) phytohemagglutinin-stimulated human peripheral blood lymphocyte conditioned medium (PHA-LCM). Colony numbers were determined by inverted microscopy in duplicate cultures after 12 days of incubation. A CFU-GM-derived colony was defined as an aggregate of ≥40 cells with characteristic morphology.

Cell cycle determination. Hydroxyurea, an S-phase-specific cytotoxic, was used to determine hematopoietic progenitor cell cycle status. An aliquot of adherent depleted peripheral blood mononuclear cells was incubated in a-medium containing 2% (vol/vol) AB serum and 10 mmol/L hydroxyurea for 1 hour at 37°C. Hydroxyurea-exposed and control cells were then cultured for BFU-E,
CFU-GM, and CFU-Meg under conditions providing maximal growth stimulation for each hematopoietic line. The decrement in the number of colonies grown from hydroxyurea-exposed cells relative to control cells represented the fraction of progenitors in cell cycle.

Statistical analyses. Megakaryocyte ploidy distributions were compared by Pearson's chi-square test of independence of distributions.13

RESULTS

Table 1 presents the clinical characteristics of the five patients with ET who were evaluated. All had sustained platelet counts >700,000/μL, which could not be attributed to other causes. The clinical course was uncomplicated in two of the patients, whereas two experienced episodes of gastrointestinal bleeding for which a specific etiology could not be found. Five years earlier, the fifth patient had had a large soft tissue hemorrhage. Her platelet count was subsequently maintained at <1,000,000/μL with intermittent busulfan therapy, and she experienced no further thrombohemorrhagic complications. None of the other four patients received any cytotoxic therapy prior to the initial culture studies.

Table 2 shows the characteristics of megakaryocyte colony growth from the five patients with ET. Patients 2 and 4 were studied on two separate occasions—3 months apart. Patient 2 received a course of busulfan between the two evaluations, whereas patient 4 was clinically stable off therapy. Concentrations of peripheral blood CFU-Meg were substantially increased relative to normal in four of the five patients (patients 1, 2, 3, and 5, Table 2). The CFU-Meg concentration in patient 2 became elevated only after the course of busulfan, whereas that in patient 4 was consistently normal (Table 2).

Unstimulated megakaryocyte colony growth was markedly increased in three patients (patients 2, 3, and 5). However, in all evaluable patients, colony growth was further augmented by the addition of aplastic canine serum to the cultures (Table 2). Because patient 5 had confluent unstimulated megakaryocyte colony growth at all plating cell concentrations tested, the effect of adding aplastic canine serum could not be determined.

We previously demonstrated that in normal individuals cultured megakaryocyte colony size increases with increasing aplastic canine serum concentration.16 This relationship was preserved in ET (data not shown). The maximum mean megakaryocyte colony size determined in the ET cultures exceeded normal in two patients (patients 2 and 3), was below normal in one patient (patient 1) and was within the normal range in patient 4 (Table 2). There was no correlation between circulating CFU-Meg concentration and abnormality in colony size.

Megakaryocyte ploidy distributions were determined after 12 days of culture, at which time ploidy values in normal cultures reach their maxima. Mean ploidy in normal cultures also varies biphasically with aplastic canine serum concentration, reaching a peak of 13.2 N at an aplastic serum concentration of 2.5% (vol/vol) (Fig 1).10 All patients with ET, had flattening of this normally biphasic mean ploidy curve. Ploidy varied minimally with aplastic canine serum concentration, and mean ploidy values were consistently below normal, reaching maxima that averaged only 8.4 N

Table 2. Characteristics of Megakaryocyte Colony Growth in Essential Thrombocytopenia

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of Megakaryocyte Colonies (per mL Peripheral Blood)</th>
<th>Maximum Mean Colony Size (Cells/Colony)</th>
<th>CFU-Meg in Cycle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstimulated</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>(range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7 ± 3 (0-23)</td>
<td>101 ± 17 (59-175)</td>
<td>22 ± 2 (19-25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22 ± 2 (21-23)</td>
</tr>
<tr>
<td>Patient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>238</td>
<td>14</td>
</tr>
<tr>
<td>2a</td>
<td>27</td>
<td>139</td>
<td>37</td>
</tr>
<tr>
<td>b</td>
<td>133</td>
<td>215</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>&gt;284</td>
<td>35</td>
</tr>
<tr>
<td>4a</td>
<td>14</td>
<td>130</td>
<td>22</td>
</tr>
<tr>
<td>b</td>
<td>29</td>
<td>127</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>&gt;3,000</td>
<td>&gt;3,000</td>
<td>C</td>
</tr>
</tbody>
</table>

NT, not tested; C, data could not be determined due to confluence of colonies.

In patients 2 and 4, a and b refer to two separate evaluations—3 months apart (described in text).
cultures of normal CFU-Meg. Table 4 compares the ploidy culture. In all three patients, the day 12 ploidy sponding decreases in 32 N and 64 N megakaryocytes. serum. As compared with normal individuals, all ET patients grams for independence of distributions'5 with the Bonferroni correction for the level of significance).

from these three patients on days I 2 distributions determined 4 on day 21 was higher than we have ever observed in culture. The increase in mean ploidy in patient 4 were determined serially on days 12, 15, 21 of culture. The increase in mean ploidy, were normal in three of the five ET patients. A substantial were determined using peripheral progenitor cells from three patients (patients 1, 3, and 4) progenitor cell-derived megakaryocytes determined over extended culture intervals. Megakaryocyte ploidy distributions were determined serially on days 12, 15, 18, and 21 of culture. In all three patients, the day 12 ploidy distributions from the two separate culture studies were similar (Table 3 v Table 4, day 12). However, with culture durations extending >12 days, patient 4 manifested a steady and substantial increase in mean ploidy, whereas the mean ploidy values from patients 1 and 3 were essentially unchanged (Fig 2). The mean ploidy of the colony megakaryocytes from patient 4 on day 21 was higher than we have ever observed in cultures of normal CFU-Meg.

Table 3 summarizes the hematopoietic progenitor cell growth abnormalities identified in the five ET patients. The largest numbers of abnormalities were observed in patients 3 and 5. The clinical courses of both of these patients have been complicated by bleeding, and their platelet counts have required control using cytotoxic agents. The other patients demonstrated fewer abnormalities and their clinical courses have tended to be more indolent. However, a strict correlation between the clinical course of ET and the characteristics increase in CFU-GM concentration occurred in two patients, one of whom also had a significant increase in BFU-E (Fig 3). In all patients, BFU-E-derived colony formation was not seen in the absence of erythropoietin and unstimulated colony growth by CFU-GM was normal, averaging six colonies/5 x 10^5 mononuclear cells. The percentages of nonmegakaryocytic progenitors (BFU-E and CFU-GM) sensitive to hydroxyurea exposure were variable, ranging from 0% to just over 50%. These data differed inconsistently from normal [normal values: BFU-E, 14% ± 11% (± SD); CFU-GM, 23% ± 11% (± SD)], and no definite trends were identifiable.

Table 4. Influence of Culture Duration on Ploidy Distributions of Progenitor Cell-Derived Megakaryocytes in Essential Thrombocythemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Days of Culture</th>
<th>No. of Megakaryocytes Evaluated</th>
<th>Ploidy Class*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>100</td>
<td>2 N 4 N 8 N 16 N 32 N 64 N</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>100</td>
<td>9 30 37 24 19 0</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>100</td>
<td>11 37 40 24 19 0</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>104</td>
<td>27 24 31 14 9 4</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>104</td>
<td>21 0</td>
</tr>
<tr>
<td>21</td>
<td>100</td>
<td>11 31 33 21 4 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>100</td>
<td>1 1 15 25 31 24</td>
</tr>
</tbody>
</table>

All cultures were performed using 2.5% aplastic canine serum (vol/vol) as the source of Meg-CSA.

*Values are percentages of total cells in each ploidy class.

Fig 2. Serial determinations of the geometric mean ploidy of colony megakaryocytes determined over extended culture intervals of 12 to 21 days. Cultures were established using peripheral blood progenitors from three patients with ET. Aplastic canine sera at a concentration of 2.5% (vol/vol) served as the source of Meg-CSA. Each patient was evaluated in a single experiment, and every ploidy determination represents the mean of measurements from a minimum of 100 megakaryocytes (except for patient 4 on day 15, when only 38 megakaryocytes could be identified in the culture plate).
of in vitro hematopoietic progenitor cell growth could not be established from this small sample.

**DISCUSSION**

ET is a myeloproliferative disorder increasingly recognized in the clinical setting. Despite the demonstration that ET is a clonal disorder originating in the pluripotent hematopoietic stem cell, the pathogenesis of the thrombocytosis remains obscure. Previous investigations evaluating its pathophysiology have been limited in number and hampered by a lack of suitable methodology. Initial observations were obtained from the morphologically recognizable megakaryocyte compartment and focused on changes in bone marrow megakaryocyte volume and ploidy (estimated by nuclear lobe counts). Increased peripheral blood concentrations of other hematopoietic progenitors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Megakaryocyte progenitors</td>
<td></td>
</tr>
<tr>
<td>1. Increased concentration in the peripheral blood</td>
<td>+ ± + 0 +</td>
</tr>
<tr>
<td>2. Increased mitotic development</td>
<td>0 + + 0 ?</td>
</tr>
<tr>
<td>3. Substantially increased unstimulated colony growth</td>
<td>0 ± + 0 +</td>
</tr>
<tr>
<td>4. Failure to achieve normal extent of polyploidization by day 12 of culture</td>
<td>+ + + ?</td>
</tr>
<tr>
<td>5. Excess polyploidization after prolonged culture intervals</td>
<td>0 NT 0 + NT</td>
</tr>
<tr>
<td>Increased peripheral blood concentrations of other hematopoietic progenitors</td>
<td></td>
</tr>
<tr>
<td>1. CFU-GM</td>
<td>0 0 0 0 +</td>
</tr>
<tr>
<td>2. BFU-E</td>
<td>0 0 0 0 +</td>
</tr>
</tbody>
</table>

*Present, +; absent, 0; could not be determined, ?, not tested, NT; increased only on second of two separate evaluations, ± (described in text and Table 2).
patients. Indeed, after prolonged culture intervals, this was
the case in one of the patients studied (patient 4). Such a late
peak in polyploidization in vitro is atypical since colony
megakaryocytes cultured from normal individuals achieve
maximum endoreduplication after intervals of 12 to 15
days.10 In the other two ET patients evaluated over extended
culture intervals (patients 1 and 3), mean ploidy values were
unchanged over time. Thus, in vitro megakaryocyte progeni-
tor cell development in ET appears to be characterized by
defective endoreduplication, resulting in the production of
either abnormally low or high ploidy megakaryocytes.

The association of low ploidy megakaryocytes with ET is
supported by the recent investigation by Knecht and Streu-
li.28 By immunostaining bone marrow cells for intracytoplas-
mic factor VIIIAg, they showed that patients with ET have
an abnormally high percentage of small megakaryocytes
(20 μm).28 Although the investigators interpreted these
small megakaryocytes as immature, such cells may have
been mature but of low ploidy. Furthermore, in a recent
study from our laboratory, the bone marrow megakaryocyte
ploidy distribution histogram from one of five ET patients
demonstrated an abnormally high percentage of low (ie, 2 N
and 4 N ploidy) megakaryocytes.19

In the current investigation, each of the five ET patients
manifested at least one hematopoietic progenitor cell growth
abnormality in vitro that potentially contributed to the
thrombocytosis observed in vivo. These included: (a) an
expanded megakaryocyte progenitor cell pool, (b) progenitor
cell-derived megakaryocyte development autonomous of or
hypersensitive to low levels of Meg-CSA, (c) above-normal
 colony size, and (d) excessive endoreduplication (Table 5).

We did not evaluate circulating levels of Meg-CSA in our
patients because previous studies by Gewirtz et al22 and our
laboratory4 indicated that inappropriately high levels of
serum Meg-CSA were not present in patients with ET.
However, more recent data from Komatsu et al suggest that
detectable levels of Meg-CSA may indeed be present in the
plasma of some ET patients.27 The discrepancy with serum
data may be explained by the routine contamination of
serum with platelet-derived inhibitors of in vitro megakaryo-
cytopoiesis.17,29

Overall, our investigation indicates that the pathogenesis
of the thrombocytosis associated with ET is a result of a
defect at the level of the pluripotent stem cell. This defect
frequently results in an abnormal expansion of the mega-
karyocyte progenitor cell compartment in vivo coupled with
abnormal mitotic and endomitotic megakaryocyte develop-
ment demonstrable in vitro.

ACKNOWLEDGMENT

We gratefully acknowledge the gift of the Leitz microscope, MPV
compact photometer, Epson HX-20 computer from the Masonic
Grand Lodge Charities of Rhode Island and the laboratory
equipment purchased by the Ivor Mason Cancer Research Fund. The
word processing assistance of Diane Matuszek is greatly appre-
ciated.

REFERENCES

1. Dameshek W: Some speculations on the myeloproliferative
 syndromes [editorial]. Blood 6:312, 1951
2. Fialkow PJ, Faget GB, Jacobson RJ, Vaidya K, Murphy S:
Evidence that essential thrombocythemia is a clonal disorder with
3. Ogawa M: Annotation: Cellular mechanisms of myeloprolifera-
Steinmann L, Erust C, Fialkow PJ: Polycythemia vera. Further in
vitro studies of hematopoietic regulation. J Clin Invest 66:1363,
1980
5. Mazur EM, Hoffman R, Chasis J, Marchesi S, Bruno E:
Immunofluorescent identification of human megakaryocyte colonies
6. Vainchenker W, Bouquet J, Guichard J, Breton-Gorius J: 
Megakaryocyte colony formation from human bone marrow precur-
1981
in the serum of patients with disorders of thrombopoiesis that
stimulates formation of megakaryocytic colonies. New Engl J Med
305:533, 1981
9. Mazur EM, South K: Human megakaryocyte colony stimulat-
ing factor in sera from aplastic dogs: Partial purification, character-
ation, and determination of hematopoietic cell lineage specificity.
10. Arriaga M, South K, Cohen JL, Mazur EM: Interrelation-
ship between mitosis and endomitosis in cultures of human mega-
11. Murphy S, Iland H, Rosenthal D, Laszlo J: Essential throm-
bocthyemia: An interim report from the polycythemia vera study
group. Semin Hematol 23:177, 1986
of human megakaryocytes in culture, in Levine RF, Williams N,
Levin J, Evtat BL (eds): Megakaryocyte Development and Func-
tion. Liss, New York, 1986, p 193
13. Ruch F: Principles and some applications of cytofluorometry,
in Wied GL, Bahr GF (eds): Introduction to Quantitative Cyto-
14. Dainiak N, Cohen CM: Surface membrane vesicles from
mononuclear cells stimulate erythroid stem cells to proliferate in
culture. Blood 60:583, 1982
Winston, New York, 1963, p 578
Invest 48:963, 1969
17. Branhög I, Ridell B, Swolin B, Weinfield A: Megakaryocyte
quantifications in relation to thrombokinetics in primary thrombocy-
18. Thiele J, Funke S, Holgado S, Choritz H, Georgii A: Mega-
karyopoiesis in chronic myeloproliferative diseases: A morphometric
evaluation with special emphasis on primary thrombocythemia.
Anal Quant Cytol 6:155, 1984
19. Mazur EM, Lindquist DL, de Alarcon PA, Cohen JL:
Evaluation of bone marrow megakaryocyte ploidy distributions in
individuals with normal and abnormal platelet counts. J Lab Clin
Med 111:194, 1988
20. Croizat H, Amato D, McLeod DL, Eskinazi D, Axelrad AA:
Differences among myeloproliferative disorders in the behavior of

From www.bloodjournal.org by guest on October 21, 2017. For personal use only.
Growth characteristics of circulating hematopoietic progenitor cells from patients with essential thrombocytemia

EM Mazur, JL Cohen and L Bogart