Spontaneous Megakaryocytic Colony Formation Does Not Discriminate between Essential Thrombocythemia and Polycythemia Vera

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Laboratory detection of spontaneous growth of colony-forming unit-megacaryocytes (CFU-MK), allowing us to distinguish essential thrombocythemia (ET) from reactive thrombocytosis, is therefore useful for the diagnostic of this myeloproliferative disorder. Whether CFU-MK assays allow us to discriminate at least partly between ET and other myeloproliferative disorders such as polycythemia vera (PV) remains, however, to be established. To gain insights about this point, we have performed CFU-MK cultures from bone marrow cells of patients diagnosed with ET ($n = 42$) or PV ($n = 50$) using a standardized collagen-based serum-free method. Spontaneous growth of CFU-MK was similarly detected in both 40/42 patients with ET and 47/50 patients with PV. These data suggest clearly that the CFU-MK assay is useful to detect not only ET, but also PV, but fails to discriminate, even partly, between these two myeloproliferative disorders. Am. J. Hematol. 81:554–556, 2006. © 2006 Wiley-Liss, Inc.

Key words: colony-forming unit megacaryocytes; essential thrombocytemia; polycythemia vera; endogenous growth

INTRODUCTION

Essential thrombocythemia (ET) is a clonal myeloproliferative disorder (MPD) resulting in an excess platelet production [1]. Its diagnosis remains largely a diagnosis of exclusion of other MPDs such as polycythemia vera (PV) and of secondary reactive causes of thrombocytosis [2]. Interestingly, endogenous growth of colony-forming unit-megacaryocytes (CFU-MK) from bone marrow cells, i.e., in vitro development of CFU-MK without the addition of growth factors, is thought to be useful for the diagnosis of ET since it is present in ET, but not in reactive thrombocytosis [3,4]. In addition, spontaneous CFU-MK growth may occur less frequently in PV than in ET, suggesting that CFU-MK cultures may contribute to discriminate at least partly between these two MPDs [3,5–7]. To gain insights about this point, we have performed CFU-MK cultures from bone marrow cells of 92 patients suffering from ET or PV using a standardized serum-free collagen-based medium.

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PATIENTS AND METHODS

Patients

Ninety-two patients seen at diagnosis in the hematology and medicine departments for PV (50 patients) or ET (42 patients) were included in this study. The average ages of ET and PV patients were 56.3 (range 21–81) and 59.6 years old (range 18–75), respectively. PV was diagnosed according to the PVSG criteria, whereas diagnosis of ET was based on bone marrow biopsy, lack of criteria of reactive thrombocytosis or

PV, and no Philadelphia chromosome or BCR-ABL detection.

CFU-MK Detection

Growth of CFU-MK in the presence or absence of cytokines (thrombopoeitin, IL-3, and IL-6) was analyzed by plating 1×10^5 bone marrow mononuclear cells/culture dish in a collagen-based medium Megacult (StemCell technologies, Meylan, France) according to the instructions of the manufacturer of the medium. After a 14-day culture period, CFU-MK growth, defined as an aggregate of at least 3 cells, was visualized through immunostaining with anti-human CD41 monoclonal antibody and an alkaline phosphatase detection system.

Statistical Analysis

Data were analyzed using the χ^2 test or the nonparametric Mann–Whitney U test.

RESULTS AND DISCUSSION

Data from CFU-MK cultures are indicated in Table I. Spontaneous CFU-MK growth was found for 87 (40 ET, 47 PV) of 92 patients, indicating high sensitivities of this test for patients suffering from ET (95%) or PV (94%). The number of positive CFU-MK assays, however, did not statistically differ between PV and ET ($P = 0.79$). Similarly, the percentage of endogenous CFU-MK growth and the absolute count of spontaneous and cytokine-dependent megakaryocytic colonies were not significantly different in ET and PV patients. As previously reported [3,4,6], we failed to detect endogenous CFU-MK growth in healthy individuals or in patients with reactive thrombocytosis (data not shown). Taken together, our data suggest clearly that the CFU-MK assay is useful to detect not only ET, but also PV, without discriminating between these two MPDs, however.

In contrast to our present data, various studies [3,5,6] have reported a lower percentage of positive CFU-MK assays in PV (ranging from 33 to 55%) compared to that occurring in ET. This may be linked to the variety of CFU-MK culture methods used, which is well known to be a source of difficulty for CFU-MK assay standardization [1]. In the present study, we used a commercial standardized collagen-based serum-free assay fully validated for CFU-MK assays [4], allowing us to prevent cell lysis occurring with other semi-solid media such as plasma clots, and which does not contain serum-containing factors like TGF-β inhibiting megacaryocytopoiesis [8]. In addition, it is noteworthy that we have read CFU-MK cultures after 14 days of incubation; by contrast, Dobo et al. [5] and Mi et al. [6] have retained a shorter culture time (8 to 10 days) for analyzing CFU-MK growth, which may explain their lower rate of positive CFU-MK assays in PV patients. The fact that some of the studies reporting a low incidence of endogenous CFU-MK growth in PV have been performed with a relative small number of patients [3,6] may also contribute to the differences between the data of these studies and our present results obtained with a higher number of patients suffering from PV.

The very high percentage of PV patients exhibiting spontaneous CFU-MK growth suggests that the recently described JAK2 mutation found in the vast majority of patients with PV [9] may be involved not only in colony-forming unit-erythroid growth, but also in CFU-MK growth. About half of the patients with ET, however, failed to exhibit acquired mutation of JAK2 [1], suggesting that distinct molecular factors, yet undetermined, are probably implicated in the abnormal growth of CFU-MK in these subjects.

TABLE I. Growth of CFU-MK in ET and PV Patients

	ET ($n = 42$)	PV ($n = 50$)
Positive CFU-MK assay[a]	40/42 (95.2%)	47/50 (94.0%)
Number of endogenous CFU-MK/dish	12.0 ± 15.9	9.8 ± 13.6
Number of cytokine-dependent CFU-MK/dish	61.2 ± 47.0	54.2 ± 44.7
Percentage of endogenous CFU-MK growth[b]	26.4 ± 38.5	28.7 ± 39.2

[a]Defined by the presence of at least one endogenous megacaryocytic colony.
[b]Defined as the ratio number of endogenous megacaryocytic colonies × 100/number of cytokine-dependent megacaryocytic colonies.

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