Identification and Conditions for Selective Expression of Megakaryocytic Markers in Friend Erythroleukemia Cells

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Friend murine erythroleukemia cells (MELCs) have been re-evaluated in terms of their nature and potential pathways of differentiation. MELC induced with 5 mmol/L hexamethylenemisbacetamide (HMBA), in addition to expression of known markers of the erythroid phenotype, were also found to exhibit traits of the megakaryocytic lineage. Erythroid differentiation was shown by the typical synthesis and accumulation of hemoglobin (Hb); megakaryoblastoid differentiation of MELCs upon induction was shown by increased specific activity of acetylcholinesterase (AChE). Incubation of MELCs with 5 mmol/L HMBA in RPMI supplemented with 1% fetal calf serum (FCS) (instead of the usual 5%), induced cells to selectively express high levels of AChE (up to ~170 mU/mg protein) with little activation of Hb synthesis (less than 5% B+ cells). The increase in AChE levels was a general phenomenon affecting the whole cell population and approached its maximum within 3 days of incubation with the inducer. Subsequently, MELCs become committed to terminal division, undergoing growth arrest and expression of the megakaryocytic phenotype even after the removal of HMBA.

There were no appreciable changes of basal AChE levels in MELCs that were either made resistant to HMBA or treated with 0.1 mmol/L hemin that activated differentiated erythroid cells to selectively express high levels of AChE (up to a170 mU/mg protein) with little activation of Hb synthesis (less than 5% B+ cells). The increase in AChE levels was a general phenomenon affecting the whole cell population and approached its maximum within 3 days of incubation with the inducer. Subsequently, MELCs become committed to terminal division, undergoing growth arrest and expression of the megakaryocytic phenotype even after the removal of HMBA.

Induced MELCs always underwent AChE increase that was more or less pronounced depending on the low or high serum content in culture, respectively. Conversely, Hb expression was permitted only when MELCs were transferred in the late phase or at the end of commitment from low to high serum media. Variations of FCS content in culture media proved to be a simple and reliable approach to change the MELC response to inducers and to modulate expression of either megakaryocytic or mixed erythromegakaryocytic phenotype. These findings suggested that MELC might be considered, at least, as a bipotential model of differentiation to be used for studies on regulation of either megakaryocytic or erythroid markers and on competition between the two hematopoietic lineages. In this regard, it was intriguing that AChE levels attained under selective induction (low serum) were always higher than under conditions allowing coexpression of both AChE and Hb (high serum). Moreover, MELCs were also found to bind the specific rat-anti-mouse platelet monoclonal antibody 4A5. Flow cytometry experiments have shown that both uninduced and especially PMA-treated MELCs were prompted to express 4A5-positivity, which increased progressively during 4 days of culture. On the contrary, HMBA-induced cultures showed no appreciable binding of the antibody within the same period. Despite differences in the modulation of AChE and platelet antigen, both of these megakaryocytic markers were expressed constitutively and independently from Hb. The fact that Hb, AChE, and platelet antigen could be elicited, whether alternatively or concurrently, in the bulk of MELC population was strongly against the supposed unipotentiality of these cells.

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FRIEND MURINE erythroleukemia cells (MELCs) are a virus-transformed cell line believed to consist of undifferentiated erythroid precursors that can be induced to terminal maturation in vitro. The most efficient inducers known are synthetic compounds such as hexamethylenebisacetamide (HMBA) and dimethyl sulfoxide (DMSO), which promote the arrest of cell growth and lead to synthesis of hemoglobin (Hb). These and other changes appear to resemble those occurring at some stages of normal erythroid differentiation; consequently, MELCs have been widely used as a model to investigate molecular events of murine erythropoiesis.

However, data from several laboratories have shown that MELCs may express traits of leukopoietic differentiation as well as enzymes including esterases, thromboxane A2 synthetase, and true acetylcholinesterase (AChE), not typically encountered in murine erythrocytes. Moreover, it has been reported that AChE, a specific marker of rodent megakaryocytes, was constitutively expressed in undifferentiated MELCs and secreted into the medium. In addition, AChE was found to be also greatly enhanced in either HMBA- or DMSO-induced cultures, preceding, and accompanying classical Hb accumulation.

These findings support the hypothesis that MELCs, rather than being purely erythroid in nature, might simultaneously coexpress markers of distinct hematopoietic lineages, as was reported to occur in the human erythroleukemic cell lines K562 and HEL. The characteristic expression and regulation of AChE in MELCs may provide evidence for a megakaryocyte-like program that resides and actively operates in these cells.

To further investigate the regulation of erythroid and non-erythroid markers in MELCs, we attempted to identify and dissect molecular events linked to induced differentiation. Specifically, we were set out to verify whether increases in Hb and AChE, denoting the expression of erythroid and megakaryocytic traits, respectively, might be separably induced. This would provide additional evidence for the exis-
tence of independent pathways through which MELC phenotypes might be modulated and augment the general knowledge of this well-known yet not thoroughly explained cell model of differentiation.

MATERIALS AND METHODS

MELCs (745/A strain and subclones) were propagated in RPMI medium (GIBCO Laboratories, Paisley, Scotland) supplemented with 5% fetal calf serum (FCS; Boehringer Mannheim, Mannheim, Germany) at 37°C in a 6% CO2 humidified atmosphere. Subclones, obtained by limiting dilution of the main strain, were tested for response to inducers by the benzidine test15 for Hb and benzidine-positive (B') cells were scored by means of a light microscopy. Subclones used for these experiments were chosen on the basis of their high susceptibility to synthesize and accumulate Hb upon induction (70% to 80% B').

Inducers used were HMBA; DMSO (Janssen Chimica, Beerse, Belgium); hemin (Sigma Chemical Co, St Louis, MO). Phorbol 12-myristate 13-acetate (PMA) was also used as an inhibitor of induced terminal erythroid maturation.16 The standard protocol for induced differentiation of MELCs involves incubation of cells (at a density of 5 to 15 × 10⁶/mL) for 5 days in the presence of a suitable agent in RPMI supplemented with 5% FCS. Changes in either length of incubation, cell density, percentage of FCS in the medium or other culture conditions are specified in the legends to figures and tables. Clones of MELCs made resistant to HMBA were obtained by repeated passage of the cell line in the presence of the inducer. Acquired HMBA-resistance was assessed by measuring either growth rates or benzidine positivity upon induction with 5 mmol/L HMBA.

Rates of MELC growth were determined by estimating the increase in cell number within 0 to 6 days after inoculation. Cell viability was assessed by trypan blue exclusion. Protein concentrations were determined according to Bradford.17

Extraction of MELCs was performed on cells washed twice with PBS and suspended in 0.5 to 2.5 mL of 0.87% NH₄Cl containing 0.2% Triton X-100. Cell suspensions were sonicated at 100 Watts for 30 seconds on ice and then centrifuged at 11,000 rpm for 1 minute in a Minifuge (Beckman Instruments, Irvine, CA). The resulting supernatants were used for assays. AChE activity was determined spectrophotometrically at 405 nm according to the DTNB method18 using 5 mmol/L acetylthiocholine iodide (Boehringer Mannheim) as the substrate. Activity was expressed in milliunits, i.e., the amount of enzyme that catalyzes the conversion of 1 nanomole of acetylthiocholine per minute, at 25°C.

Hb concentrations were measured from the visible absorption spectra typical of oxyhemoglobin. The A₄₁₅ values were multiplied by 0.1221 to give Hb concentration in milligrams per milliliter.

Cytocentrifugates were obtained onto a glass slide by centrifugation of 1 mL of cell suspension with 10 to 20 × 10⁶ cells at 1,500 rpm for 10 minutes (Minifuge GL; Heraeus-Christ, Osterode am Harz, Germany), at 20°C. Glasses were air-dried and then stained with AChE according to the copper-thiocoline method.19 The reagent was prepared fresh every time and incubated with the slides for about 4 hours at room temperature. Cells were counterstained with Papanicolau's solution 1a (Merck, Darmstadt, Germany).

Specificity to the murine megakaryocyte lineage was evaluated with the aid of the specific rat-antimurine platelet monoclonal antibody (MoAb) 4A5. This antibody, although not tested with other tissues, was previously characterized as a selective label of murine blood platelets and marrow megakaryocytes.19 Cells (~ 1 × 10⁵) were incubated in phosphate-buffered saline (PBS) added with 1% bovine serum albumin (BSA) for 60 minutes at 0°C with a preparation of biotin-conjugated 4A5 IgG (20 μg/mL). Cells were then incubated with streptavidin-R phycoerythrin conjugate for 60 minutes at 4°C. Positive cells were identified by flow cytometry on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) after gating for nonspecific fluorescence measured in control tubes (cells processed as experimental ones but without biotin-4A5).

RESULTS

The standard protocol adopted for MELC commitment to terminal maturation involved the incubation of cells in RPMI plus 5% FCS and 5 mmol/L HMBA (or 1.5% DMSO). Under these conditions the expression of both Hb and AChE was stimulated to various degrees depending on the clone used. Induced cells showed a progressive reduction in growth rates and concurrent accumulation of Hb, approaching a maximum by the fifth day of culture. Percentages of hemoglobinized cells, shown by the benzidine test, rose from 5% to 2.5% to 10%, which diverged significantly, whereas values within the same group were not different from each other. In turn, AChE at 5% did not differ significantly from enzyme content of either the 5% or 10% group.

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within the range of 1% to 5% FCS; subsequently it leveled off and remained fairly constant around 100 μg/mg protein. AChE specific activity increased sharply to yield \( \approx 170 \) mU/mg protein at 1% and 2.5% FCS. At higher serum concentrations, enzyme levels were partially reduced compared with the maximum, yet they were maintained at over 100 mU/mg protein. HMBA-induced MELCs incubated at 1% FCS were exceedingly high in AChE whereas Hb content was very low (10 μg Hb/mg corresponding to less than 5% \( \text{B}^+ \) cells). This finding indicated that under conditions of restricted FCS content, the megakaryocyte-like and erythroid differentiation processes of MELCs might be dissociated. None of the control cultures (without HMBA) showed appreciable changes in either AChE and Hb at any of the FCS concentrations used (1% to 10%), implying that the increase in AChE was due to MELC interaction with the inducer. Statistical analyses of data (see legend for detailed comments) has shown that Hb levels at 1%, 2.5%, and 5% were significantly different from each other. With regard to AChE, the levels attained at 1% to 2.5% diverged significantly from those at 7.5% to 10% to confirm a slight inhibitory effect of

![Image](http://www.bloodjournal.org)
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At various intervals, aliquots of cell suspensions were processed immediately for AChE assay (○, --) to follow the time course of enzyme increase. Other aliquots of cell suspensions were used for commitment experiments as follows. Cells, washed free of HMBA-containing medium, were resuspended with an equal volume of fresh RPMI plus 1% FCS and incubated up to day 4 when cultures were assayed for AChE specific activity (○, --). Cultures that were treated for 3 days with HMBA and then transferred to fresh media without the inducer were followed up to day 6. At each interval, MELCs were counted in a Burker chamber to assess increase in cell number and calculate PDLs, reported in the text. Results are the means ± SD of three separate experiments.

To determine the time-course of AChE increase upon induction, MELCs were incubated for various intervals in the presence of 5 mmol/L HMBA and 1% FCS in RPMI. At the times indicated, aliquots of MELCs were taken and processed to determine AChE specific activity (Fig 4). During the first day of incubation with HMBA, AChE was maintained close to the basal level. Thereafter, AChE increased steadily up to 90 mU/mg protein on day 3 and approached a maximum by day 4. Along with the time-course experiment, other aliquots of the MELC suspension were withdrawn separately and cells were transferred to a fresh medium without HMBA. MELCs were then processed on the following days of incubation to ascertain whether enzyme levels of HMBA-induced cultures were affected by removal of the inducer. Results reported in Fig 4 showed that during the first 2 days of incubation with HMBA, the transfer of cells to fresh medium without inducer did not change enzyme levels attained just before HMBA removal. Rather, the cells that were withdrawn after 3 days of incubation with HMBA and transferred to fresh medium without inducer, showed a significant burst in enzyme activity approaching 150 mU/mg protein. Such a high level of AChE was attained just after 24 hours of incubation in fresh medium and was maintained constantly for the following 2 days.

During the 6 days of incubation in culture, variations in cell number were also monitored, at each interval, with the aid of a Burker chamber. MELC growth in the presence of HMBA was found to continue at a reduced rate, as compared with control, up to day 3 (population doublings [PDLs] calculated for HMBA-treated and controls were 2.3 and 3.1, respectively). Thereafter, induced cells underwent growth arrest (PDL, 0.39 over the last 4 days of incubation) independently of either the presence of HMBA or density at which they were inoculated into fresh medium. These results were in keeping with a process of HMBA-induced commitment of MELCs to terminal maturation through the megakaryocytic rather than erythrocytic pathway.

Effects of FCS content in the medium on expression of differentiation markers in HMBA-induced MELCs have been investigated further. MELCs were preincubated at 1% FCS with 5 mmol/L HMBA for 2 days, that is a time insufficient for commitment. Thereafter, the cell suspension was split into two parts that were spun down and resuspended with fresh medium containing 5 mmol/L HMBA and either 1% or 5% FCS. Three days later, MELCs maintained at 1% FCS with the inducer showed the typical large increase in AChE to reach a mean specific activity of 98.4 mU/mg protein, whereas B’ cells were less than 5% (Fig 5). On the

Fig 4. Time-course of AChE increase and commitment of MELCs by 5 mmol/L HMBA at 1% FCS in the culture medium. Cells were initially seeded at a density of 0.2 × 10⁶/mL in the presence of HMBA.

Fig 5. AChE and Hb levels of MELCs preinduced at 1% FCS and transferred to either 1% or 5% FCS inducer-containing media. Cells (10⁶/mL) were preincubated in RPMI plus 1% FCS and 5 mmol/L HMBA for 2 days. Subsequently, the cell suspension was divided into two parts and centrifuged. Cells were then resuspended with an equal amount of fresh medium containing 5 mmol/L HMBA and either 1% or 5% FCS and incubated into new dishes for 3 days. At the end of incubation, cells were subjected to the benzidine test and assayed for AChE activity as reported above. Results were the means ± SD of three different experiments.
contrary, HMBA-induced MELCs that were transferred from 1% to 5% FCS developed into a hemoglobinized population (74% B+ cells) also exhibiting increased AChE specific activity of ≈66 mU AChE/mg protein. Similar results were obtained when cells were transferred after a preincubation with HMBA at 1% FCS for 3 days, which is the time required for full commitment. These findings seemed to indicate that low and high serum could modulate cell differentiation toward either megacytogenic or mixed erythromegakaryocytic phenotype by acting in the late phase of commitment or even after this has occurred.

Results of Table 1 are a summary of AChE modulation under various culture conditions. Of particular interest were data showing that PMA added to MELC cultures 2 days before HMBA did not prevent AChE from increasing at either 1% or 5% FCS. However, erythroid differentiation in MELCs exposed to HMBA in the presence of 5% FCS (83% B+ cells) was efficiently repressed by the PMA treatment (48% B+ cells). Additionally, (1) MELCs made resistant to HMBA did not respond to the inducer and maintained both Hb and AChE at basal levels; (2) concentrations of HMBA required to trigger AChE increases were lower at 1% than 5% FCS; and (3) DMSO was a less effective inducer of AChE than was HMBA.

Additional evidence for the expression of megakaryocytic markers in MELCs was provided by flow cytometry experiments using a biotin-conjugated preparation of the MoAb 4A5, which was specifically raised against a murine platelet antigen. Results of Fig 6 showed the time-course of 4A5 positivity in cultures maintained at 1% FCS and treated in the absence and in the presence of either PMA (100 ng/mL) or HMBA (5 mmol/L). One day after seeding all cultures were virtually unreactive with 4A5, as shown by the coincidence of specific and nonspecific signal, apart from PMA-treated cells that exhibited a partial positivity. At day 2, approximately half of the control cells were found to bind the antibody, whereas most of PMA-treated MELCs were already progressed towards 4A5 positivity; in turn, HMBA-treated cells still showed no appreciable binding. A clear 4A5 positivity of both control and PMA-treated MELCs was attained at day 4, as shown by the shift on the right of the gray area and by the fact that 4A5-positive cells were broadly distributed over the entire population (data not shown). HMBA-treated cells remained substantially negative even at day 4. These results indicated that the platelet antigen recognized by the antibody 4A5 is constitutively expressed by uninduced MELCs during growth in culture. The addition of PMA seemed to accelerate, as compared with control, the antigen expression. On the contrary, the incubation of MELCs with HMBA prevented MELCs from expressing the platelet antigen within a 4-day period. It is worth mentioning that flow cytometry results have been confirmed by experiments performed with several freshly-isolated subclones of MELCs to rule out any problem of dealing with mixed populations.

**DISCUSSION**

During the past several years evidence for the potential megakaryocytic nature of MELCs has been accumulating. This includes (1) the ability of MELCs to incorporate serotonin; (2) the constitutive and induced expression of AChE together with the typical intracellular distribution of the enzyme; (3) the active extracellular release of AChE as it occurred in murine megakaryocytes and the inhibitory effect exerted by exogenous AChE on MELC growth; and (4) the finding that activin A-treated MELCs expressed thromboxane A2 synthetase, an enzyme occurring in murine megakaryocytes but not in erythrocytes.

Megakaryocytes and erythrocytes are believed to share common precursors and there are evidences that most erythroblast and megakaryoblastic cell lines express markers specific to both the erythrocytic and megakaryocytic lineages. Therefore, it is not surprising that MELCs, in addition to erythroid markers, could also show traits of the megakaryocytic lineage as it was reported previously by us and very recently confirmed by the effects of activin A/EDF on erythroid and megakaryocytic differentiation in the

**Table 1. Effects of Various Culture Conditions on AChE Specific Activity and Benzidine Positivity of MELCs**

<table>
<thead>
<tr>
<th>Culture Conditions</th>
<th>AChE Specific Activity (mU/mg protein)*</th>
<th>B+ Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELCs, uninduced</td>
<td>18.8 ± 7 (9)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 1 mmol/L HMBA</td>
<td>24.7 (1)</td>
<td>7.3</td>
</tr>
<tr>
<td>MELCs + 2.5 mmol/L HMBA</td>
<td>57.6 (1)</td>
<td>23.6</td>
</tr>
<tr>
<td>MELCs + 5 mmol/L HMBA</td>
<td>117.4 ± 30 (5)</td>
<td>83.7</td>
</tr>
<tr>
<td>MELCs + 1.5% DMSO</td>
<td>92.4 ± 32 (5)</td>
<td>66.8</td>
</tr>
<tr>
<td>MELCs + 0.1 mmol/L hemin</td>
<td>24.8 ± 7 (2)</td>
<td>76.9</td>
</tr>
<tr>
<td>MELCs + 100 ng/mL PMA</td>
<td>22.9 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 100 ng/mL PMA + 5 mmol/L HMBA</td>
<td>141.9 ± 20 (4)</td>
<td>48</td>
</tr>
<tr>
<td>HMBA-resistant MELCs, uninduced</td>
<td>8.7 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>HMBA-resistant MELCs + 5 mmol/L HMBA</td>
<td>19.8 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs, uninduced</td>
<td>30.8 ± 6 (7)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 1 mmol/L HMBA</td>
<td>62.4 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 2.5 mmol/L HMBA</td>
<td>104.1 ± 9 (3)</td>
<td>4.3</td>
</tr>
<tr>
<td>MELCs + 5 mmol/L HMBA</td>
<td>141.6 ± 42 (7)</td>
<td>4.3</td>
</tr>
<tr>
<td>MELCs + 1.5% DMSO</td>
<td>66.7 ± 33 (3)</td>
<td>5.8</td>
</tr>
<tr>
<td>MELCs + 0.1 mmol/L hemin</td>
<td>25.0 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 100 ng/mL PMA</td>
<td>37.9 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 100 ng/mL PMA + 2.5 mmol/L HMBA</td>
<td>138.2 ± 7 (2)</td>
<td>=1</td>
</tr>
<tr>
<td>MELCs + 100 ng/mL PMA + 5 mmol/L HMBA</td>
<td>137.9 ± 4 (2)</td>
<td>=1</td>
</tr>
<tr>
<td>HMBA-resistant MELCs, uninduced</td>
<td>14.7 (1)</td>
<td>=1</td>
</tr>
<tr>
<td>HMBA-resistant MELCs + 5 mmol/L HMBA</td>
<td>12.1 (1)</td>
<td>=1</td>
</tr>
</tbody>
</table>

* AChE specific activity was determined after 4 days of incubation and values were the means ± SD. Number of experiments is shown in parentheses.

† PMA was added 2 days before incubation with HMBA.

‡ HMBA-resistant MELCs were obtained as described in Materials and Methods.

EDF on erythroid and megakaryocytic differentiation in the
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Day 1    Day 2    Day 4

Fig 6. Time course of 4A5 positivity in control and either PMA- or HMBA-treated MELCs, as determined by flow cytometry. MELCs from a resting culture were diluted up to 1 x 10^6/mL in RPMI with 1% FCS in the absence (A) or presence of either PMA (100 μg/L) (B) or HMBA (5 mmol/L) (C). At days 1, 2, and 4 after seeding, the cells were harvested, washed with PBS containing 1% BSA, incubated (1 x 10^6 cells) with biotin-conjugated 4A5 (20 μg/mL) followed by incubation with streptavidin-R phycoerythrin conjugate, and then analyzed as reported in the Materials and Methods. The specific (with 4A5 antibody) and nonspecific signal (without 4A5 antibody) have been indicated as gray and empty areas, respectively.

MELC line F55. Nevertheless, the practice of using MELCs as an erythroid system alone with little attention paid to markers other than Hb, has hampered a full understanding of this model. Under culture conditions routinely used for MELC differentiation, the synthesis of Hb was always found to be triggered, whereas AChE expression could be either increased (with 5 mmol/L HMBA or 1.5% DMSO as the inducers) or maintained at basal levels (with 0.1 mmol/L hemin). In the former situation, induced MELCs were committed to terminal division and could be regarded as a mixed phenotype coexpressing both megakaryocytic and erythroid markers. In the case of MELCs induced with hemin, these cells were not committed to terminal division and behaved as practically pure erythroid precursors.

We have shown herein that by decreasing FCS in the culture medium, HMBA-induced MELCs were induced to resume their resident megakaryocytic program with negligible activation of the erythrocyte differentiation pathway. The presence of HMBA was necessary to promote the AChE increase; therefore, it appears that there is a true commitment to terminal maturation involving the expression of differentiated functions and contemporary arrest of cell growth.

It is not clear why low serum concentrations channel induced MELCs toward the megakaryocytic rather than erythroid phenotype. However, there are two possible explanations that we are presently evaluating. The first is that the inducer might interact differently with fast-growing (at 5% FCS) or slow-growing (at 1% FCS) cell populations. Two facts might support this assumption: (1) the typical commitment of MELCs by HMBA was reported to require at least one cycle of cellular replication to take place; and (2) as we have previously observed, variations of AChE levels in uninduced MELCs appeared to be inversely related to rates of cellular replication. The second conceivable explanation is that restricted serum conditions may lead to a decrease in factors that act preferentially as inhibitors of megakaryocyte differentiation, like it was reported for transforming growth factor β78,29 and platelet factor 4.30 The inhibitory activity of these factors might be shown by the fact that HMBA-induced MELCs shifted from a plain megakaryocytic (very high AChE and negligible Hb) to a mixed erythromegakaryocytic phenotype (high AChE and high Hb) upon the transfer of cells, whether partially or fully committed, from 1% to 5% serum media (see results of Fig 5). It remains to be established whether, in addition to the lack of physiologic inhibitors that would allow HMBA to selectively turn on the
of murine platelet antigen in MELCs. Owing to the little information available on murine megakaryocytic markers and lack of probes for their detection, it is difficult and probably too early to compare megakaryocytic potentialities of MELCs with that of human erythroleukemic cell lines that also express both erythroid and megakaryocytic traits. At any rate, AChE and murine platelet antigen of MELCs were largely expressed in the bulk of MELC population. On the other hand, expression of some of the megakaryocytic features in pluripotential HEL, K562, and TF, were reported to vary within a wide range and to often involve a limited number of cells.13,24

Ultimately, AChE levels in HMBA-induced MELCs approached values of ∼150 to 200 mU/mg protein, which is more than 35% of the enzyme activity present in mature murine megakaryocytes.10 Further, we have shown that MELCs constitutively express a murine platelet antigen during growth in culture. In contrast, the amount of Hb accumulated in fully differentiated MELCs did not usually exceed 110 μg/mg protein, which is below 15% of the Hb content in mature erythrocytes. We also have data (Paoletti F, manuscript in preparation) showing that a small yet significant percentage of polyploid cells occurred spontaneously in MELC cultures and that a large part of the population, upon particular treatments, can undergo a large increase in ploidy without any activation of erythroid functions. Taken together, all these facts would imply that MELCs are a much more complex system than previously believed. Particularly, MELCs, because of their clear and spontaneous megakaryocytic nature, might be paradoxically considered more closely related to precursors of megakaryocytes than of erythrocytes. We believe that the identification of suitable conditions for alternatively inducing either megakaryoblastoid and/or erythroid markers in MELCs may be of help in further understanding of this model and the complex interplay between the erythrocytic and megakaryocytic lineages.

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