Characterization of a New Megakaryocytic Cell Line: The Dami Cell

By Sheryl M. Greenberg, David S. Rosenthal, Tammy A. Greeley, Ramana Tantravahi, and Robert I. Handin

A new human megakaryocytic cell line (Dami) has been established from the blood of a patient with megakaryoblastic leukemia. The Dami cells grow primarily in suspension with a doubling time of 24 to 30 hours. By light and electron microscopy, the Dami cells range in size from 12 to 120 μm in diameter and have lobulated nuclei characteristic of megakaryocytes. At least 99% of the cells react with monoclonal antibodies against platelet glycoproteins (GP) Iib and IIb/IIIa, and glycoporphin. The cells do not react with antibodies against lymphoid, monocyte, granulocyte, or macrophage antigens. Thirteen percent of the cells become polyploid, spontaneously achieving >4N DNA ploidy levels. In response to phorbol myristate acetate (PMA), the proportion of cells with ploidy levels greater than 4N increased threefold and could be separated into discrete ploidy groups. PMA also increased the expression of GPIb, the GPIIb/IIIa complex, and von Willebrand factor. Cytogenetic analysis revealed a human male hyperdiploid karyotype with a modal chromosome number of 54 to 64 and several consistent clonal chromosomal abnormalities. These included a partial deletion of chromosome 5 and a translocation involving chromosome 3. In contrast to other megakaryocytic cell lines in which only a small portion of the cells express the megakaryocytic phenotype, nearly all of the Dami cells express platelet glycoproteins. Thus, the Dami cells provide a superior model in which to study human megakaryocyte biochemistry and differentiation.

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MATERIALS AND METHODS

Case history. The cell line was derived from the peripheral blood of a 57-year-old man with megakaryoblastic leukemia. The patient was originally diagnosed as having myeloid metaplasia with myelofibrosis in 1975. In 1983, he was treated with hydroxyurea (HU) because of progressive splenic enlargement and weight loss. Two years later he developed left upper quadrant pain and fever and underwent splenectomy. The spleen weighed 5,041 g and had extensive extramedullary hematopoiesis with myeloid, erythroid, and megakaryocytic elements. Following splenectomy, his platelet count rose to 1,830,000/μL and WBC count to 110,000/μL with megakaryocytic fragments and megakaryoblasts identified in the blood. Over the next 24 months he received several courses of chemotherapy that included various combinations of cytosine arabinoside and daunorubicin but failed to enter hematologic remission. Blood was taken for cell analysis and initiation of cell culture when his white cell count was 50,000/μL with 63% megakaryoblasts. The patient died the next month, 13 years after the initial diagnosis and 1 year after conversion to acute megakaryocytic leukemia.

Cell culture. A heparinized blood sample was obtained, with the patient’s consent, in December 1986. Samples were diluted fourfold with Hanks’ balanced salt solution (HBSS), and fractionated on discontinuous two-step Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) gradients with densities of 1.050 and 1.085 g/mL. The cells harvested from the interface between the two layers of Percoll were washed twice in HBSS and suspended in 10% horse serum in Iscove’s modified Dulbecco’s medium (GIBCO, Grand Island, NY) containing 1% phytohemagglutinin-stimulated leukocyte-conditioned medium (PHA-LCM) in plastic tissue culture flasks. The cells were subcultured as necessary. No feeder cells were used. Where indicated, PMA, a tumor-promoting phorbol ester, was added to a final concentration of 5 × 10⁻⁸ mol/L, and dimethyl sulfoxide (DMSO) was used at a final concentration of 1.25%. For quantitative measurement of von Willebrand factor (vWF) antigen in the conditioned medium, Dami cells were cultured with 1% Nutridoma-HU (Boehringer-Mannheim Biochemicals, Indianapolis) and in the absence of exogenous vWF sources such as horse serum and PHA-LCM. Quantitation of vWF was kindly performed by Dr Bruce Ewenstein by inhibition enzyme-linked immunoassay (ELISA).

Ultrastructural studies. Cells were harvested for ultrastructural studies after 6 months of continuous culture. Cells were centrifuged at 200 g for five minutes and washed with phosphate-buffered saline.
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(PBS) before preparation for electron microscopy studies essentially according to the method of Schmit and Codognoto. Briefly, the cells were fixed in 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.2) at ambient temperature for one hour, post-fixed in 1% osmium tetroxide containing 1% potassium ferricyanide, dehydrated with ethanol and propylene oxide, and embedded in epon. Thin sections of selected areas were stained with uranyl acetate followed by lead citrate and examined by a Philips EM 300 electron microscope.

Cytogenetic analysis. Exponentially growing cells were exposed to colcemid (0.1 μg/mL) (GIBCO) for 30 minutes and washed by centrifugation. The cell pellet was resuspended in a hypotonic solution of 75 mmol/L KCl for ten minutes at ambient temperature, centrifuged, and fixed in freshly prepared methanol-glacial acetic acid (3:1 v/v), which was changed twice. Aliquots of the cell suspension were dropped onto cold wet slides and allowed to air-dry.

Slides were Q-banded by staining in an aqueous solution of quinacrine mustard (Sigma Chemical St Louis) by using published protocols. Thirty well-spread and banded metaphases were photographed by using a Leitz fluorescence microscope equipped with an Orthomat camera. Chromosome analysis was done from photographic prints. The chromosome abnormalities are described according to the ISCN nomenclature.

Ploidy analysis. Control and PMA-treated cells were cultured for four days. The cells were then washed twice with HBSS and suspended in nuclei isolation medium (NIM), a calcium- and magnesium-free HBSS containing 0.2% bovine serum albumin, 0.4% Nonidet P-40, and 10 mmol/L HEPES, pH 7.4, at a concentration of 2 × 10⁷/mL. RNase (54 Worthington U/mL) and an equal volume of NIM buffer containing 25 μg/mL propidium iodide were added. The suspensions were placed in an ice bath for at least 30 minutes before flow cytometric analysis on a Becton Dickinson Flow Cytometry Analyzer equipped with the appropriate excitation (long pass, 400 nm; band pass, 485/20 nm) and emission (band pass, 575/26; long pass, 570 nm) filters. The ploidy distribution of the cells was compared with freshly prepared lymphocytes and propidium iodide 2 N standard beads (Flow Cytometry Standards Corp., Research Triangle Park, NC).

Indirect immunofluorescence assay. The expression of surface antigens was analyzed by using a panel of monoclonal antibodies recognizing epitopes on hematopoietic cells. Cultured cells were washed in HBSS, adjusted to a concentration of 1 × 10⁷/mL, and incubated for 30 minutes at 4°C with equivalent protein concentrations (2 μg/10⁶ cells) of monoclonal antibodies against platelet glycoprotein (GP) Ib (6D1) and the GPIIb/IIIa complex (7E3 and 10E5) (the kind gifts of Dr Barry Coller, Stony Brook, NY), myeloid and monocytic cells (MY9), or monocytic/macrophagic/granulocytic cells (Mol), which were purchased from Coulter Immunology, Hialeah, FL. Mouse IgG or IgM was used for the determination of negative or background staining. After two washes by centrifugation in HBSS, the cells were incubated with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG or IgM at a dilution of 1:100 for 30 minutes at 4°C. After an additional centrifugation in PBS containing 1% paraformaldehyde, the number of cells reactive with each antibody was determined by flow cytometry on a Becton Dickinson Flow Cytometry Analyzer (Becton Dickinson, Mountain View, CA) equipped with the appropriate excitation (long pass, 400 nm; band pass, 458/20 nm) and emission (band pass, 575 nm) filters. At least 5,000 cells were analyzed for each antibody, and the experiment was conducted every 3 months after cell proliferation began (12 weeks after the initial seeding of the patient’s fractionated blood). The proportion of “positive” cells was determined by comparison to cells reacted with nontissue mouse IgG or IgM or with the FITC-conjugated secondary antibody alone as a negative control.

Northern blot analysis. Total RNA was prepared from 5 × 10⁶ cells by guanidine hydrochloride extraction and subjected to electrophoresis in 1% agarose denaturing gels as previously described. Equal amounts of total RNA (10 μg) from each sample underwent electrophoresis and were then transferred electrophoretically to ZetaProbe filters (Bio-Rad Laboratories, Richmond, CA). The complementary DNA probe for GPIb (the kind gift of Drs Jose Lopez and Jerry Roth, University of Washington, Seattle) was radiolabeled by using the method of random hexanucleotide priming and used for hybridization. Filters washed at high stringency (15 mm sodium chloride, 1.5 mm sodium citrate, 0.1% SDS, 55°C) were exposed to XAR film (Kodak Laboratories, Rochester, NY) for autoradiography.

RESULTS

Cell culture and growth characteristics. The cells proliferated very slowly for the first few weeks after initiation of culture but began to proliferate rapidly within 12 weeks, with a doubling time of 24 to 30 hours. Currently, the cells require subculturing twice weekly. The new cell line, designated Dami, adheres to tissue culture plasticware until the cell density begins to increase, after which they grow both as adherent cells and in grape-like clusters in suspension (Fig 1). There is considerable variation in cell size, with a range of 12 to 120 μm in diameter. Typically, even the adherent cells were rounded, although cytoplasmic protrusions or pseudopods were often observed in viable cells. Greater than 95% of the cells excluded trypan blue and were thus considered viable; the nonviable cells were usually rounded and/or fragmented. The Dami cells have maintained a population doubling time of 24 to 30 hours during exponential growth and have been maintained in continuous culture for a year and a half. Culture in the presence of PMA results in a substantial decrease in proliferation, increased adherence to tissue culture plasticware, and increased cell spreading. They are free from Epstein-Barr virus (EBV) or mycoplasma.

Morphologic examination. At the time the megakaryoblastic cells were isolated from the patient's blood, his white cell count was 54,000/μL (corrected for nucleated RBCs) and the platelet count was 21,000/μL. The differential count
revealed 2% neutrophils, 20% lymphocytes, 1% monocytes, 2% basophils, 6% myelocytes, 5% promyelocytes, and 61% blast forms. In addition, there were 27 nucleated RBCs and 37 megakaryocytic fragments per 100 WBCs. Wright-Giemsa staining of blood smears (Fig 2A and B) demonstrates the high percentage of blasts. The immature cells had irregular nuclei and budding cytoplasm. Large platelet forms, megakaryocyte fragments, nucleated red cells, and polyploid megakaryoblasts were also noted.

The cultured cells were examined periodically. Cells obtained at 12 weeks, shortly after the cells began proliferating, were frozen and are compared with smears of cells maintained in continuous culture for 1 year (Fig 3A and B). Both preparations were similar and demonstrate polyploid blast cells with frequent budding of the cytoplasm as well as smaller, less differentiated blast cells. In each case, the cytoplasm was basophilic and devoid of granules.

**Transmission electron microscopy.** The Dami cell line was heterogeneous, with cells having the characteristics of megakaryoblasts or immature megakaryocytes. The predominant cells contained a lobulated nucleus, multiple prominent nucleoli, and a high nuclear-cytoplasm volume ratio (Fig 4). The cytoplasm contained prominent smooth endoplasmic reticulum, extensive Golgi complex, and numerous large mitochondria. Only rare granules were seen. Cytoplasmic budding was frequently observed, but no demarcation membranes were noted.

**Cytogenetic analysis.** Cytogenetic studies of peripheral blood and a bone marrow aspirate in 1983, when the patient presented with splenic enlargement, revealed a normal 46,XY karyotype. This cell line was established from a peripheral blood sample obtained in 1986, and after 8 months in culture, the Dami cell line had a near triploid chromosome number with a range of 54 to 64 chromosomes. Several clonal structural abnormalities were observed in the 20 cells analyzed (Fig 5). Chromosomal aberrations of the major clone are summarized in Table 1. There were trisomies of 16 of the autosomal chromosomes. There were also deletions of specific regions on chromosomes 4, 6, and 20. A
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large number of reciprocal translocations were identified. The four completely defined translocations involved chromosomes 1 and 6, 3 and 6, 4 and 8, and 5 and 17. A majority of the cells had normal X and Y chromosomes.

Ploidy analysis. Nuclei isolated from exponentially growing Dami cells were predominantly 2N and 4N (45.0% and 38.0%, respectively) with a small proportion (12.8%) containing DNA equivalents of 8N or greater (Fig 6). An equal number of cells from each sample (5,000) was analyzed. When viewed by a fluorescence microscope, these polyploid nuclei did not appear to be aggregates of isolated nuclei. To confirm the ploidy distribution measured by using isolated nuclei as well as to demonstrate that the polyploid nuclei were not merely aggregates, another method that uses intact cells treated with hypotonic citrate to facilitate nuclear staining by propidium iodide was used. The ploidy distribution was confirmed, and no cell aggregates were detected by fluorescence microscopy. Cultures treated with the tumor-promoting phorbol ester PMA for either four or ten days had a greater proportion of cells containing complements of DNA greater than 4N (22.7% and 36.7%) than did control cells, thus suggesting maturation of the cells. In addition, these polyploid Dami cells fell neatly into distinct groups containing 8N, 16N, 32N, and 64N levels of DNA. DMSO had a negligible effect on the Dami cell ploidy levels. Other hematopoietic cell lines, including HEL, K562, U937, and HL-60 cells, did not increase the ploidy distribution of the cells when incubated with PMA.

Cell surface markers. The results of cell surface antigen analysis are summarized in Table 2. Nearly all of the cultured cells expressed platelet GPIb and GPIIb/IIIa as well as glycophrin, HLA class I antigen, and the myeloid antigen recognized by the MY9 antibody. Less than 10% of the cells reacted with the FITC-conjugated secondary antibody alone and were referenced as negative staining. Lymphoid antigens were uniformly absent, as were those of monocytes, granulocytes, and macrophages.

Indirect immunofluorescence histograms of control and PMA-treated cultured Dami cells demonstrated an increased surface expression of platelet GPIb and the GPIIb/IIIa complex in response to PMA (Fig 7). Nonspecific antibody binding did not increase concomitantly, as shown by the similar fluorescence distributions of control or PMA-treated cells reacted with anti-T1, an antibody against a T-cell antigen present throughout T-cell maturation (Fig 7, top panels); cell reactivity with the secondary, fluorescently tagged antibody alone was similar to that with anti-T1 (data not shown).

SDS-PAGE and Western blot analysis. Electrophoresis of control, DMSO-, and PMA-stimulated Dami cell lysates and the lysates of cells stimulated with both reagents in SDS–polyacrylamide gels demonstrates that the Dami cells express a normal-sized platelet GPIb of 170 Kd (Fig 8) that, when reduced, decreases in size to 140 Kd, the same size as the platelet GPIbα chain (Fig 8). This suggests that the Dami cells may also express the GPIbβ chain (which is translated from a different messenger RNA). PMA stimulation increases the number of molecules of GPIb per cell when equivalent numbers of cells are subjected to electrophoresis (Fig 8), thus confirming the increased reactivity with the monoclonal antibody against GPIb that was demonstrated in Fig 7.

Northern blot analysis. Hybridization of Northern blot filters with radiolabeled cDNA probes shows the presence of the 2.4-kb mRNA for GPIbα in Dami cells (Fig 9). A comparison of equivalent amounts of total RNA from control, DMSO-, and PMA-stimulated cells as well as RNA from cells stimulated by both reagents demonstrates that PMA but not DMSO enhances the expression of GPIb mRNA and that the increased surface expression of GPIb is due, at least in part, to an increase in the level of the specific GPIb message. The level of expression is maximal at least 48 hours after the cells are subcultured or after PMA is added.
demonstrates the presence of the intracellular precursor forms of vWF (260 and 275 Kd) as well as the fully processed (220-Kd) form of vWF. The pattern is identical to that seen in endothelial cells and normal guinea pig megakaryocytes. In contrast to GPIb, which is only enhanced by PMA, the addition of either PMA or DMSO enhances vWF expression (Fig 10).

**Analysis of vWF.** Dami cells washed free of and cultured in the absence of exogenous sources of vWF but in the presence of PMA and/or DMSO constitute secrete vWF antigen. Quantitation of secreted vWF by inhibition ELISA demonstrates that both PMA and/or DMSO increase the amount of secreted antigen about threefold (Fig 10). Western blot analysis of lysates from control and stimulated cells demonstrates the presence of the intracellular precursor forms of vWF (260 and 275 Kd) as well as the fully processed (220-Kd) form of vWF. The pattern is identical to that seen in endothelial cells and normal guinea pig megakaryocytes. 

**Table 1. Cytogenetic Analysis of Dami Cells After 8 Months in Continuous Culture**

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>54-64, XY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomies</td>
<td>+ 1, + 2, + 3, + 4, + 5, + 6, + 8, + 11, + 12, + 13, + 15, + 17, + 19, + 20, + 21, + 22</td>
</tr>
<tr>
<td>Deletions</td>
<td>4(q25), 6(q21), 20(q12)</td>
</tr>
<tr>
<td>Completely defined translocations</td>
<td>t(1;6)(p13;q21)</td>
</tr>
<tr>
<td></td>
<td>t(3;6)(p13;q16)</td>
</tr>
<tr>
<td></td>
<td>t(4;8)(q12;p11)</td>
</tr>
<tr>
<td></td>
<td>t(5;17)(q11;p11)</td>
</tr>
<tr>
<td>Partially defined translocations</td>
<td>t(1;7)(p21;??)</td>
</tr>
<tr>
<td></td>
<td>t(2;10)(10;7)(q21;p14;q23;??)</td>
</tr>
<tr>
<td></td>
<td>t(3;7)(q26;??)</td>
</tr>
<tr>
<td></td>
<td>t(8;7)(p11;??)</td>
</tr>
<tr>
<td></td>
<td>t(9;7)(11)p12;21;p14;??;p15)</td>
</tr>
<tr>
<td></td>
<td>t(9;7;22)(p24;??;p13)</td>
</tr>
<tr>
<td></td>
<td>t(11;21)(q11;q22)</td>
</tr>
<tr>
<td></td>
<td>t(15;7)(p11;??)</td>
</tr>
<tr>
<td></td>
<td>t(18;7)(p11;??)</td>
</tr>
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<td></td>
<td>t(19;7)(p11;??)</td>
</tr>
<tr>
<td></td>
<td>t(19;7)(p13;??)</td>
</tr>
</tbody>
</table>
Indirect immunofluorescence assays were performed as described in the text. "Negative" denotes <10% positive cells. Values represent the averages of three separate determinations.

**Table 2. Surface Antigen Characterization of the Dami Cells**

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Positive Cells (%)</th>
<th>Antibody Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6D1</td>
<td>95.7</td>
<td>Platelet GP1b</td>
<td>17</td>
</tr>
<tr>
<td>7E3</td>
<td>94.7</td>
<td>Platelet GP1b/IIIa</td>
<td>18</td>
</tr>
<tr>
<td>10E5</td>
<td>93.4</td>
<td>Platelet GP1b/IIIa</td>
<td>19</td>
</tr>
<tr>
<td>B1</td>
<td>Negative</td>
<td>Immature and mature B cells</td>
<td>20</td>
</tr>
<tr>
<td>T1</td>
<td>Negative</td>
<td>Immature and mature T cells</td>
<td>21</td>
</tr>
<tr>
<td>T11</td>
<td>Negative</td>
<td>Immature and mature T cells</td>
<td>22</td>
</tr>
<tr>
<td>Mo1</td>
<td>Negative</td>
<td>Pan-myeloid cells</td>
<td>23</td>
</tr>
<tr>
<td>My9</td>
<td>84.1</td>
<td>Multipotent progenitors</td>
<td>24, 25</td>
</tr>
<tr>
<td>W6/32</td>
<td>95.0</td>
<td>HLA class I antigen</td>
<td>26</td>
</tr>
<tr>
<td>Glycophorin A</td>
<td>88.6</td>
<td>Glycophorin A</td>
<td>27</td>
</tr>
</tbody>
</table>

Indirect immunofluorescence assays were performed as described in the text.

"Negative" denotes <10% positive cells. Values represent the averages of three separate determinations.
Fig 6. Flow cytometric analysis of propidium iodide-stained nuclei. Exponentially growing Dami cells were solubilized with a Nonidet P-40-containing buffer and the nuclei stained with propidium iodide. Freshly prepared lymphocytes were used as 2N standards. Five thousand cells were analyzed for each sample. The first major peak is representative of the proportion of cells in the population with a normal 2N complement of DNA. The subsequent peaks represent the proportion of cells with ploidies of 4N, 8N, 16N, 32N, and greater levels of DNA as shown on a log scale of the DNA content.

DISCUSSION

Human hematopoietic cell lines developed from the blood of patients with acute leukemias have become useful tools to study lymphoid, myeloid, and erythroid differentiation. However, there are no permanent cell lines that uniformly express the megakaryocyte phenotype to use for similar studies of megakaryocyte differentiation. HEL cells, derived from the peripheral blood of a patient with Hodgkin's disease who later developed erythroleukemia, only express the platelet membrane protein GPIb in a minority of the cells, and this protein has been reported to be smaller in size than the protein normally found in platelets. Ogura et al have reported a human megakaryocytic leukemia cell line (MEG-01) established from the bone marrow of a patient with Philadelphia chromosome-positive chronic myelogenous leukemia. Although this cell line uniformly expresses platelet GPIIb/IIIa, like the HEL line, it has limited expression of GPIb, and it reacts with a monoclonal antibody against another hematopoietic lineage, the B cell. Sledge et al have reported a cell line (EST-IU) derived from a patient with acute megakaryoblastic leukemia. These cells uniformly express both GPIb and the GPIIb/IIIa complex but have a finite life span of 6 months. The LAMA-84 cell line only expresses GPIIb/IIIa on 50% of the cells and has no GPIb, and the K562 erythroleukemia line has only limited expression of GPIIb/IIIa and no detectable GPIb or vWF.

We have characterized a new human cell line, Dami, developed from the peripheral blood of a patient with acute megakaryoblastic leukemia. A small portion of the cell population spontaneously achieves ploidy levels greater than 4N, a property characteristic of normal megakaryocytes. The cultured cells have maintained the initial megakaryo-

Fig 7. Indirect immunofluorescence flow cytometric analysis of cultured Dami cells. The top panels represent cells reacted with anti-T1, a pan-T cell monoclonal antibody, as negative controls and plotted on a log scale of fluorescence intensity. The two lower panels represent cells reactive with 6D1 and 10E5, monoclonal antibodies against platelet GPIb and GPIIb/IIIa, respectively. The left panels represent control cells cultured in the absence of any inducing reagents; the right panels represent cells cultured in the presence of PMA. Five thousand cells were analyzed for each sample.

Fig 8. SDS-PAGE and Western blot analysis of GPIb. Lysates of 2.5 × 10⁶ control and DMSO- and/or PMA-induced Dami cells were subjected to electrophoresis in each lane of an SDS-PAGE gel and subsequently transferred electrophoretically to nitrocellulose filters. The nitrocellulose filters were stained with rabbit polyclonal antibodies against platelet GPIb and then with an ¹²⁵I-labeled, affinity purified, goat antirabbit IgG.
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Fig 9. Northern blot analysis of Dami cell RNA. Ten micrograms of total RNA prepared from control and DMSO- and/or PMA-induced Dami cells at 2, 24, and 48 hours after the initiation of culture and addition of inducing reagents was subjected to electrophoresis in 1% agarose formaldehyde gels and electrophorosed onto Zetaprobe filters. The filters were hybridized with a 32P-labeled cDNA probe for GPIb, washed at high stringency, and autoradiographed.

Fig 10. vWF analysis. Conditioned medium of Dami cells cultured for 0, 2, and 5 hours in the absence of exogenous sources of vWF and after four days in the presence of PMA and/or DMSO where indicated were assayed by inhibition ELISA. (Inset) Western blot analysis of intracellular vWF in lysates of 2.5 x 10^7 Dami cells after culture in the presence of PMA and/or DMSO as indicated. Nitrocellulose filters were stained with polyclonal rabbit anti-vWF antiserum and 125I-labeled, affinity-purified goat antirabbit IgG and autoradiographed.

...and platelets do not express this antigen. However, the MY9 antigen has been previously identified on megakaryoblasts, and it has been postulated that this antigen appears transiently on megakaryocytes during early differentiation.35 The Dami cells do not express the lymphoid or myeloid-specific antigens usually identified on blast cells and thus appear to be a pure population of megakaryocyte-like cells. This does not exclude the possibility that other chemical differentiation-inducing reagents may stimulate the expression of proteins characteristic of nonmegakaryocytic hematopoietic lineages in this cell line.

The only nonmegakaryocytic characteristic determined to date is the expression of glycoporphin A. However, this is a common occurrence in most of the other lines with megakaryocytic potential, ie, HEL, K562, and LAMA-84.7,8,36

This bipotential quality is not well understood but is presumed to represent hematopoietic cells at a very early stage of differentiation. Since the presence of platelet and erythrocydl markers on the same cell has not been carefully examined in normal marrow, it is possible that this phenotype also occurs in early committed bone marrow cells of the megakaryocyte and erythroid lineages.

The Dami cell line has a very complex karyotype with consistent numerical and structural abnormalities. A small population of cells appears to have double the chromosome number of the original complement and may represent the polyploid nature that is characteristic of megakaryocytes as well as this cell line. The partial deletion of the long arms of chromosomes 5 and 20 have been reported in both de novo and therapy-linked myelodysplastic syndromes.37,38 The partial deletion of the long arm of chromosome 5 observed in this cell line is produced as a result of a 5:17 translocation with the associated loss of the short arm of chromosome 17. This is frequently observed in bone marrow cells of patients with myelodysplasia.39 A number of cases of acute nonlymphocytic leukemia have been reported with abnormalities of the long arm of chromosome 3.40 Simultaneous involvement of bands 3q21 and 3q26 are thought to be associated with abnormal megakaryocytopenia.40 However, only band 3q26 appears to be involved in a translocation in this cell line.

The process of megakaryocyte differentiation and maturation in normal bone marrow cells proceeds through sequential stages.41 After commitment to the megakaryocyte/platelet lineage, the committed megakaryocytic stem cell (promegakaryoblast) expresses some platelet plasma membrane proteins and begins endoreduplication. Subsequent megakaryocytic maturation involves cytoplasmic development followed by the formation of platelets. The uniform presence of platelet GPIb and the GPIIb/IIa complex, the polyploid nature of a small portion of these cells, and the relative absence of α-granules and demarcation membranes suggest that the Dami cells represent a stage of development common to normal megakaryoblasts and immature megakaryocytes. In response to the tumor-promoting phorbol ester PMA, the Dami cells become polyploid, with the cells falling into discrete ploidy groups containing 8N, 16N, 32N, and 64N amounts of DNA. In contrast, other hematopoietic cell lines with megakaryocytic properties, ie, HEL and K562, do not become polyploid in response to PMA. The expression of...
platelet plasma membrane GPIb and GPIIIa complex and of the α-granule protein vWF were also increased in response to PMA, thus suggesting a potential for further megakaryocytic differentiation.

The Dami cells resemble normal human megakaryocytes more closely than do previously reported cell lines and respond to a tumor-promoting phorbol ester by an increase in the expression of platelet proteins. Thus, the Dami cells provide a useful tool for studying specific questions related to the biochemistry of megakaryocyte differentiation, nuclear and cytoplasmic factors that might be involved in differential expression of specific genes, and the biochemical profile of early progenitor cells. Due to the difficulties inherent in the preparation of pure populations of megakaryoblasts and megakaryocytes, the Dami cell line provides a promising alternative source of cells for investigation.

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