Characterization of a Factor-Dependent Acute Leukemia Cell Line With Translocation (3;3)(q21;q26)

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A strictly factor-dependent cell line (UCSD/AML1) was established from a patient with the syndrome of multilineage acute leukemia with high platelets. The patient’s cells and the cell line karyotype were 45,XX,-7; t(3;3)(q21;q26), typical of the syndrome of acute leukemia with high platelets. The cell line expresses CD34, CD7, TdT, and myeloid (CD13, CD14, CD33) and megakaryocyte/platelet (CD36, CD41, CD42b, CDw49b) antigens. In short-term culture, UCSD/AML1 cells proliferate in response to interleukin-3 (IL-3), IL-4, IL-6, macrophage colony-stimulating factor (M-CSF), and granulocyte-macrophage CSF (GM-CSF), but not IL-1, IL-2, IL-5, or G-CSF. In long-term culture, proliferation can be sustained by GM-CSF, IL-6, or M-CSF.

CERTAIN SUBTYPES of human acute leukemias are characterized by specific clinical features and karyotype abnormalities, suggesting that genetic lesions determine the pathogenesis or phenotypic manifestations of these syndromes. One such syndrome is characterized by acute nonlymphocytic leukemia (ANLL) presenting with high or normal platelet counts and chromosome deletions or translocations involving 3q21 and/or 3q26. Clinical findings in these patients are consistent with increased and abnormal megakaryocytopenia. Because genes involved in Fe metabolism (ie, transferrin and its receptor) are located in this area of chromosome 3, and Fe deficiency is characterized by increased platelet production, it has been suggested that these genes may somehow determine the clinical features of this syndrome.

In the present studies, we describe a new, strictly factor-dependent cell line (UCSD/AML1) established from a patient with the syndrome of acute leukemia with high platelets and t(3;3)(q21;q26). These cells express megakaryocytic/platelet antigens and undergo primary proliferative responses and abnormal megakaryocytic differentiation in response to interleukin-6 (IL-6). Because IL-6 was recently shown to be an in vitro megakaryocyte maturation factor and an in vivo thrombopoietin, our findings suggest the ANLL syndrome associated with t(3;3)(q21;q26) involves cells with the ability to express megakaryocytic differentiation programs, and suggest IL-6 may play a role in the phenotypic manifestations of this disease. UCSD/AML1 cells also express antigens associated with multilineage hematopoietic progenitor cells, and display some characteristics of a second acute leukemia syndrome, CD7 positive acute leukemia with multilineage differentiation.

CASE HISTORY

The patient was a 73-year-old woman who presented to an outside hospital with progressive fatigue. She had no known exposure to cytotoxic drugs or radiation, and no prior history of a blood cell disorder. Her plasma Hb was 9.5 g/dL, platelet count 450,000/mm³, and white blood cell (WBC) count 6,800/mm³. The leukocyte differential showed 26% PMN, 9% bands, 9% metamyelocytes, 11% myelocytes, 28% lymphocytes, and 26 NRBCs/100 WBC. A bone marrow performed at an outside hospital was hypercellular and showed normal to slightly increased megakaryocytes. Many of the megakaryocytes were small in size with a single large nucleus, or had multiple separate nuclei. Blasts constituted 58% of the nonerythroid elements and ranged in size from small cells with a high nuclear/cytoplasmic ratio and indistinct nuclei to cells of medium size with a moderate nuclear/cytoplasmic ratio and 1 to 3 nuclei. There were no granules or Auer rods. Occasional dysplastic erythrocyte precursors showed nuclear/cytoplasmic dysynchrony and multiple nuclei. A karyotype performed at an outside laboratory was 45,XX, -7; t(3;3)(q21;q26). Surface marker analysis, also performed at an outside laboratory, showed a mixture of cells expressing T-cell and granulocyte/monocyte related antigens (Diagnosis, Table 1). A stain for nuclear TdT was negative.

The patient developed progressive anemia over the next month, but her platelet count remained elevated. She was treated with daunorubicin, cytospin arabinoside, and high-dose cytosine arabinoside, but achieved only a brief remission. A repeat bone marrow was performed at relapse and showed greater than 70% small blasts with a T-cell phenotype (Relapse, Table 1). A karyotype performed at University of California, San Diego was again 45,XX, -7; t(3;3)(q21;q26) but, in addition, 4 of 12 metaphases analyzed demonstrated 45,XX, -7; t(3;3)(q21q26), t(12;22)(p13q12). She started on adriamycin/vincristine/prednisone therapy, but developed refractory leukemia meningitis and died.

MATERIALS AND METHODS

Cell culture. Bone marrow cells were obtained at relapse from induction therapy for ANLL. The cells were separated on Ficoll/Hypaque and cultured at 5 x 10⁵/mL in RPMI 1640 medium with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 30 U/mL recombinant granulocyte-macrophage colony-stimulating factor.
Characterization of UCSD/AML1 cells. Surface antigens on Ficol/Hypaque separated bone marrow or the UCSD/AML1 cell line were detected by immunofluorescence staining using monoclonal antibodies (MoAbs) to the CD2, CD3, CD4, CD5, CD7, CD13, CD14, CD33, CD34, CD45 antigens (nomenclature is per reference 14), and erythrocyte glycoporphins as previously described. The platelet-associated CD41 (glycoprotein [GP] IIb/IIIa) antigen was detected using antiplatelet MoAb (DAKO Diagnostics, Santa Barbara, CA) or antibody 4F10 (reference 16; kindly provided by Dr Virgil Woods, University of California, San Diego). MoAbs to VLA-2 (12F1; reference 17) (platelet GPla; CDw49b); platelet GP Ib (CD24b) (P3; reference 18); and to platelet GPIV (CD36) (5F1; reference 19) were also provided by Dr Woods. Histochemical stains were performed using standard techniques as described. Nuclear TdT was detected by immunofluorescence staining using a specific heteroantiserum (Supertechs, Bethesda, MD). Southern blots were also performed using standard techniques, and immunoglobulin (lg) and T-cell β receptor gene rearrangements assessed using probes to the Ig JH and T-cell β receptor constant regions (Oncor, Gaithersburg, MD). Placental DNA (Oncor) served as a germline control.

Giemsa-banded karyotypes were obtained as previously described. The cells also expressed the CD13, CD14, and CD33 myeloid markers. The UCSD/AML1 cells were myeloid in nature, as they were strongly positive for acid phosphatase and nonspecific esterase, but negative for chloroacetate esterase. Surface marker analysis (Table 1) showed that UCSD/AML1 cells continued to express the CD7 T-cell-associated and CD34 primitive hematopoietic cell antigens, and remained TdT+. The cells also expressed the CD13, CD14, and CD33 myeloid antigens, and the platelet GPIIb/IIIa complex (CD41). Subpopulations expressed platelet GP Ib (CD42b), the VLA2...
The cell line karyotype was 45,XX,−7,t(3;3)(q21; q26),t(12;22)(p13;q12) in all metaphases examined. The t(3;3)(q21;q26) was apparently identical to that of the patient's cells at diagnosis and relapse, and the t(12;22)(p13; q12) was identical to a subgroup of cells present at relapse only (Fig 2).

To assess growth factor responses by UCSD/AML1 cells, log-phase cells were washed three times, plated with various concentrations of growth factors and FBS, and proliferation assessed by TdR uptake. As shown in Fig 3, UCSD/AML1 cells proliferated in response to IL-3, IL-4, IL-6, GM-CSF, and M-CSF, but showed no response to IL-1, IL-2, IL-5, or G-CSF (data not shown). The cells could be maintained as a factor-dependent cell line in GM-CSF, IL-6, or M-CSF with a doubling time of 48 to 72 hours, but could be continuously grown with IL-3 only with markedly reduced cellular viability. When GM-CSF was withdrawn, the cells ceased proliferating and 50% to 75% of cells died within 2 weeks. However, when the remaining cells were replated in GM-CSF, they rapidly recovered to their baseline growth rate (three experiments).

In other factor-dependent cell lines, growth factors synergize in stimulating cell growth. Because the cell line expressed the primitive CD34 antigen, we screened several growth factor combinations, which showed synergism in culture systems for putative early ("blast") progenitors by combining the highest concentration of a growth factor that did not stimulate cell growth with a complete dose-response curve of a second growth factor. Combinations of 1 or 10 U/mL IL-4 and GM-CSF or IL-6 showed no interaction. Similarly, G-CSF (100 U/mL) and IL-4 had no effect on IL-3-stimulated cell growth (not shown).

or platelet GPIa (CD49b), and the platelet GPIV (CD36) antigens. Southern blots showed Ig and T-cell β receptor genes to be in germline configuration (data not shown). Thus, the cell line retained characteristics of primitive hematopoietic cells expressing the CD7 and CD34 antigens and TdT, but also had characteristics consistent with GM and megakaryocytic cells.

Fig 1. Morphology of UCSD/AML1 cell line grown with FBS and 30 U/mL GM-CSF (A), or with 10⁻⁸ mol/L TPA and GM-CSF (B). Multinucleated giant cells (C) were observed under all culture conditions, but increased in number when cells were grown with 30 U/mL IL-6 and GM-CSF (see text).

Fig 2. Detail of chromosome translocations in UCSD/AML1 showing t(3;3)(q21;q26) and t(12;22)(p13;q12).
The new cell line described in the current studies retains properties of the acute leukemia with high-platelets syndrome. The cell line has a 3q21 translocation characteristic of this syndrome. Monosomy 7, present in the patient's cells and the cell line, and pre-existing myelodysplasia were also previously described as components of this syndrome. Expression of GP IIb/IIIa, CD13, and CD14 by UCSD/AML1 cells is similar to expression of platelet antigens by cells from a patient with ANLL and t(1;3)(p36;q21). Thus, UCSD/AML1 cells appear to be the first cell line to preserve properties of an ANLL syndrome associated with a specific chromosomal translocation.

The original tumor cells and the cell line also share properties with multipotent CD7+ acute leukemias. The cell line retains CD7 expression and TdT positivity. In vitro, UCSD/AML1 cells form multinucleated giant cells consistent with abnormal megakaryocytes. Virtually identical cells were observed in short-term cultures of CD7+ acute leukemia cells. Several patients with CD7+ acute leukemia described in a previous report also had normal or high platelet counts, but lacked chromosome 3 abnormalities. Although UCSD/AML1 cells differentiate into both macrophage and megakaryocyte-like cells, thus far we have been unable to induce the broad range of lymphocyte and myeloid differentiation observed in short-term cultures of CD7+ acute leukemia cells.

The ability of UCSD/AML1 cells to undergo aberrant megakaryocyte differentiation and proliferate in response to IL-6 may explain some features of the ANLL with high-platelets syndrome. The appearance of increased multinucleated giant cells after incubation with IL-6 and GM-CSF, but not IL-6 alone, suggests that UCSD/AML1 cells will be useful for studying cytokine interactions in this syndrome.

The expression of primitive hematopoietic cell, myeloid, and megakaryocytic antigens by UCSD/AML1 cells suggested these cells could undergo multilineage differentiation. When UCSD/AML1 cells were cultured with GM-CSF and TPA, they rapidly became adherent, and a few showed distinct spreading on plastic. The morphology of the cells became macrophage-like (Fig 1B), and expression of the CD14 antigen increased from 25% to 52% of cells (means of two experiments). Increased cellular adherence and spreading were also observed after long-term (2 weeks) culture with M-CSF. In the presence of GM-CSF and TPA, the number of multinucleated giant cells also increased slightly over cultures maintained in GM-CSF alone from 3% ± 1% of total cells to 8% ± 3% (means ± SE; three experiments). Identical results were obtained when cells were grown for 5 days with TPA alone, but cell viabilities were decreased.

Because IL-6 was recently shown to be a maturation factor for megakaryocytes, surface antigen expression and morphology of UCSD/AML1 cells were also examined after culture with IL-6. After 5 days of culture with GM-CSF and 30 U/mL IL-6, the number of multinucleated giant cells increased fourfold to sevenfold (18% ± 6%; N = 3), but did not increase in the presence of IL-6 alone. In contrast, immunofluorescence staining after exposure to IL-6 (not shown) or IL-6 with GM-CSF showed increased expression of three platelet-associated antigens (platelet GPIIb/IIIa complex, platelet GPIa, and platelet GPIV) (Fig 4). When cells were gated according to low-angle light scatter, increased platelet antigen expression was observed in cells of both intermediate and large size, suggesting the increase in platelet antigen expression was not restricted to multinucleated giant cells. Thus, UCSD/AML1 cells showed evidence for both macrophage and megakaryocytic differentiation.

**DISCUSSION**

The new cell line described in the current studies retains properties of the acute leukemia with high-platelets syndrome. The cell line has a 3q21 translocation characteristic of this syndrome. Monosomy 7, present in the patient's cells and the cell line, and pre-existing myelodysplasia were also previously described as components of this syndrome. Expression of GP IIb/IIIa, CD13, and CD14 by UCSD/AML1 cells is similar to expression of platelet antigens by cells from a patient with ANLL and t(1;3)(p36;q21). Thus, UCSD/AML1 cells appear to be the first cell line to preserve properties of an ANLL syndrome associated with a specific chromosomal translocation.

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**Fig 3.** Proliferation of UCSD/AML1 cells in response to hematopoietic growth factors. Cells grown with FBS and GM-CSF were washed and grown with no addition or various concentrations of the growth factors shown for 4 days. Results shown are the means of three to five experiments.

**Fig 4.** Expression of platelet-related antigens by UCSD/AML1 cells detected by immunofluorescence after culture with FBS and GM-CSF or with GM-CSF and IL-6. Representative results from one of three experiments are shown. Staining with irrelevant control antibody (—).
future studies, it will also be important to examine effects of IL-6 on proliferation and differentiation of primary tumor cells from patients with t(3;3)(q21;q26).

Whether genes involved in the t(3;3)(q21;q26) induce leukemic transformation or alter its phenotypic manifestations is at present unclear. Although it has been postulated that genes involved in cellular Fe metabolism may be involved in these translocations, Southern blots of DNA from the UCSD/AML cells showed no gross rearrangements of the transferrin receptor gene (data not shown). The establishment of the UCSD/AML cell line should allow cloning and characterization of the genes involved in this translocation.

In short-term culture, UCSD/AML cells respond to a wide variety of growth factors, including IL-6, IL-4, and MCSF, which may stimulate primitive normal marrow ("blast") progenitors, but do not directly stimulate growth of normal GM progenitors or most ANLL cells in vitro. The extent to which human factor-dependent leukemia cell lines reflect growth factor responses by primary ANLL cells is unclear. It is possible that continuous exposure to growth factors in vitro selects for cells or mutations allowing factor-independent growth in the same way culture with FBS and media alone selects for relatively factor-independent cells. UCSD/AML cells contain a t(12;22) present in only a subpopulation of the patients' cells at relapse, indicating selection in culture for this tumor cell population. The broad range of growth factor responses displayed by these cells could give these cells a proliferative advantage in vivo over their normal counterparts.

A subpopulation of UCSD/AML cells survives growth factor deprivation for extended periods. These results differ from studies using murine factor-dependent cells that require growth factors, such as IL-3, for survival and rapidly die when growth factors are withdrawn. We are currently evaluating whether growth factors contained in FBS may partially maintain UCSD/AML cell viability. This property of the cell line may allow detailed study of the nonproliferating (Go) state in factor-dependent human myeloid leukemia cells.

UCSD/AML represent a valuable addition to the limited roster of available factor-dependent, human ANLL cell lines. Establishment of cell lines from patients with other acute leukemia syndromes may provide similar insights into the pathophysiology of these acute leukemia syndromes, and the eventual definition of the roles chromosome translocations play in the establishment and maintenance of acute leukemia.

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