Identification of a Malignant Counterpart of the Monocyte-Dendritic Cell Progenitor in an Acute Myeloid Leukemia

By Frances Santiago-Schwarz, Donald L. Coppock, Alexander A. Hindenburg, and Joshua Kern

Myeloblasts derived from the peripheral blood of a patient with acute myelogenous leukemia (ORL47) were found to represent the malignant counterpart of the newly elucidated monocyte–dendritic cell colony-forming unit (mono-DC-CFU). The specific cytokine conditions required to achieve intermediate and terminal maturation of DCs and monocytic cells from these progenitors were defined. With tumor necrosis factor (TNF) + granulocyte-macrophage colony-stimulating factor (GM-CSF) + stem cell factor treatment numerous colony-like clusters developed. In contrast with normal DC development, further advancement of mono-DC-CFU and terminal DC maturation from the leukemic cells were dependent on the addition of interleukin-6. Functional and phenotypic analysis showed that the capacity to differentiate was maintained fully in the DC compartment, but only partially in the monocyte compartment, as judged by the lack of CD14 surface expression. Cells found at intermediate stages of DC development were potent stimulators of a mixed leucocyte reaction, a function usually attributed to mature DCs. As previously shown for normal DC development, antibodies to TNFα and GM-CSF blocked proliferative responses and DC growth. The importance of these observations in the classification of leukemias, normal DC development, and potential clinical strategies is discussed. © 1994 by The American Society of Hematology.

RECENT ADVANCES in stem cell biology have had tremendous impact on our understanding of the pathophysiology of hematologic malignancies. Both normal and malignant hematopoietic differentiation are multistep processes governed by cytokines. Distinct cytokines, acting alone or in synergy, initiate lineage commitment from a common multipotential progenitor cell, subsequently produce intermediate progenitors, and ultimately generate end-stage progeny. Thus, the fate of specialized progeny is not only dependent on the immediate external environment, but also on the responsive state of the precursor cell. Various malignant counterparts exist which correspond to particular stages of the lymphoid, myeloid, erythroid, and megakaryocytic series, suggesting that clonal aberrations may occur at different levels of hematologic development.

In general terms, myeloid leukemias may be defined as hematologic malignancies frozen at early stages of myeloid differentiation. As with other clonal abnormalities, self-renewal in acute leukemias is favored over differentiation, although limited normal differentiation events may occur in vivo. In vitro studies have shown that heterogeneity in the proliferative and maturation responses of myeloid leukemias is caused by arrest at different stages of hematopoietic differentiation. The current French-American-British (FAB) classification system for acute myeloid leukemias (AMLs) considers this heterogeneity and recognizes eight subgroups (M0-M7). Further heterogeneity has been described within subgroups, such as M2, to include distinct chromosomal translocations, ie, t(8:21). Notwithstanding, leukemic clones regularly fall outside these schemes, suggesting that normal equivalents of particular clones remain unidentified or unclassified. The known AML subgroups that are not defined by FAB are undifferentiated acute leukemia, M0, mixed lineage acute leukemia, and hypopcellular AML.

Cells belonging to the dendritic cell (DC) series are distinct myeloid elements that have been recognized for some time as crucial in managing the presentation of antigen to immune cells. There have been several accounts of malignancies exhibiting features of DCs. However, there has been no identification of the malignant progenitor in any of these diseases and there is currently no way of classifying DC malignancies. Some investigators have argued that a proportion of monocytic leukemias are precursors of Langerhans cells (LC, the skin component of the DC series) in LC histiocytosis (LCH), but the relationship between the monocytic and the LC pathway was not described.

We and others have recently traced the normal developmental pathway of DCs and have shown that DCs and monocytic cells develop simultaneously under the influence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF) from a common progenitor cell unit that has been termed the monocyte–DC colony-forming unit (mono-DC-CFU). In addition, we have shown that stem cell factor (SCF) in combination with TNF and GM-CSF optimizes the development of DCs from these progenitor cells (Santiago-Schwarz F, manuscript in preparation). In this study, we have exploited observations of normal DC development to recognize a malignant counterpart of the mono-DC-CFU in an AML that we designate ORL47. We show that the leukemic cells differentiate along the DC pathway and define the specific cytokine combinations required to achieve seemingly normal differentiation events. The term myelodendritic leukemia is suggested to describe this malignancy.

MATERIALS AND METHODS

Classification of the myeloid leukemia and clinical course. The leukemic cells analyzed were obtained after informed consent from a 38-year-old white woman with refractory AML. The patient presented 4 months earlier with fevers and diffuse inflammation of the left breast and rectum. Myeloblasts were detected on the peripheral smear, a bone marrow (BM) aspiration, biopsy, and a needle aspiration biopsy of the breast were performed, and the diagnosis of AML, FAB M2, with granulocytic sarcoma was made according to standard
morphologic and cytochemical criteria by two independent reviewers. The BM differential (the Wright stain is shown in Fig 1) showed 47% myeloblasts, 5% promyelocytes, 2% myelocytes, 8% metamyelocytes, 25% polymorphonuclear cells, 6% lymphocytes, 2% plasma cells, and 5% normoblasts. Sudan Black staining was strongly positive in 10% of the cells, weakly positive in 66%, and negative in 24%. Myeloperoxidase staining was strongly positive in 11%, weakly positive in 62%, and negative in 27%. α-naphthyl acetate esterase staining was strongly positive in 3%, weakly positive in 17%, and negative in 80%. Combined esterase staining showed mostly chloroacetate esterase positive (76%), and α-naphthyl acetate esterase negative (78%).

Immunophenotypic analysis of the BM aspirate (performed at the Roswell Park Memorial Institute, Buffalo, NY) showed two separate populations: a more primitive population was CD33+CD13+ (20%), whereas a more differentiated one expressed only CD13 (57%). The single expression of the T-cell markers CD2, CD3, CD7, and CD8 was less than 3%. Total CD4 expression was 32%, and, of these, only 1.13% were CD3+CD4+CD8–M and 0.16% were CD3+CD4+CD8+. B-cell marker expression, including CD10, CD19, and CD20 was less than 2%, and, natural killer (NK) (CD16, CD56) cell–associated marker expression was less than 1%. Total CD34, HLA-DR, CD11b and CD14 expression was 33%, 17%, 62%, and 3.4%, respectively.

Karyotypic analyses performed on two distinct occasions were normal. Needle biopsy of the breast yielded only scant myeloblasts and neutrophils. No bacteria were identified on the smears, and microbiologic cultures were negative.

The patient was treated with empiric broad-spectrum antibiotics that included anaerobic coverage for the presumed infection or superinfection of the breast and rectum. The leukemia was refractory to two courses of chemotherapy with cytosine arabinoside and daunorubicin. Remission was achieved with m-Amsa (amsacrine; National Cancer Institute, Bethesda, MD) and high-dose cytosine arabinoside. Four months later, the patient relapsed and died with refractory disease. Immediately before her death, she developed a granulocytic sarcoma of the tongue and required intubation. Needle aspiration of the tongue showed scant myeloblasts and neutrophils.

Enrichment of leukemic blasts. Thirty milliliters of peripheral blood (PB) was obtained by venipuncture in accordance with institutional guidelines. At the time of collection (4 months after diagnosis), the patient had not received chemotherapy for 10 weeks, and the PB white blood cell (WBC) count was 59,000/mL. WBCs were isolated from the buffy coat after centrifugation and stored in liquid nitrogen in 10% dimethylsulfoxide/90% growth medium until needed. After thawing, leukemic cells (designated ORL47) were adjusted to ~4 × 10⁶ cells/mL in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (FBS; GIBCO), 2 mmol/L glutamine, 50 IU/mL penicillin, 50 μg/mL streptomycin (FBS/RPMI) and placed in a 5% CO₂ humidified incubator at 37°C for 3 to 5 days. Nonadherent cells were then removed from culture, washed in RPMI 1640, and adjusted to 0.5 to 1 × 10⁷ cells/mL. The blast cell content after this preincubation period was greater than 80%; lymphocyte content was less than 5%, and polymorphonuclear leukocyte (PMN) content was less than 10%, as determined by Wright-stain analysis and flow cytometry.

Cytokine treatment. Recombinant cytokines were added to the leukemic blasts, either alone or in combination, and included TNFα (Knoll Pharmaceuticals, Whippany, NJ) at a final concentration of 500 U/mL; GM-CSF (Genzyme, Boston, MA) at a final concentration of 100 U/mL; SCF (Genzyme) at a final concentration of 50 ng/mL; and interleukin-6 (IL-6; Genzyme) at a concentration of 100 U/mL. The concentration for each cytokine was determined after dose-response analysis as previously described and is optimal for achieving DC development from normal stem cells.22 Mouse antihuman GM-CSF monoclonal antibody and antihuman TNFα polyclonal (Genzyme) were used at 10 μg/mL. The optimal doses of these antibodies and the effects of nonspecific controls were described in an earlier study.24

Endotoxin levels in these cultures were controlled as previously described.23 Culture vessels consisted of 24-well tissue-culture plates and two-well chamber slides (plastic Lab-Tek chamber slides;
Nunc, Naperville, IL). The leukemic cell lines HL60 and U937 and PB cells from another leukemic patient (ORL49, MS) were cultured at 2 to $3 \times 10^3$ cells/mL in parallel fashion.

Proliferative assessment of cultured cells. Growth and viability were assessed by the uptake of tritiated thymidine and by hemacytometer-assisted cell counts (Improved Neubauer; Fisher Scientific, Pittsburgh, PA). For thymidine uptake, 0.5 $\mu$Ci of $[^{3}H]$ thymidine (specific activity, 25 Ci/mmol, Amersham, Arlington, IL) was added to 100 $\mu$L of cells in microtiter plates. After incubation for 5 hours, cells were harvested using an automated sample harvester and counted in a liquid scintillation counter. Results are expressed as means of triplicate counts.

Cytochemistry. Cells in suspension were prepared for Wright-stain (Hemacolor, EM Diagnostic Systems, Fisher Scientific, Gibbstown, NJ) analysis and nonspecific-esterase (NSE) staining (a-naphthyl acetate esterase staining kit; Sigma Diagnostics, St Louis, MO) by depositing them onto slides by cytocentrifugation (Shandon, Pittsburgh, PA). Cells in chamber slides were stained in situ.

Mixed leukocyte reaction. Stimulator populations consisted of ORL47 leukemic cells grown under the conditions indicated for 24 hours in Teflon culture vials (Scientific Specialties Service, Randals-town, MD). After this period, cells were removed from culture, washed, assessed for viability by Trypan blue exclusion, and irradiated with a $^{60}$Co source (100 rads/min for a total of 2,000 rads). Varying numbers of stimulator cells were then added in triplicate to 96-well microtiter plates containing $5 \times 10^3$ responder cells (nylon wool-enriched T-cell populations prepared from normal PB) per well. After 6 days of incubation, proliferative responses were measured by the uptake of tritiated thymidine as described above.

Statistics. Where indicated, Student's $t$-test analysis was performed using a CRUNCH Interactive Statistical Package (CRISP; Crunch Software Corp, San Francisco, CA).

RESULTS

Growth of ORL47 cells. During preincubation for 3 to 5 days in FBS/RPMI, most (>80%) of the nonadherent leukemic cells retained blast morphology, as assessed by Wright-stain analysis (Fig 2). Examination by phase microcopy showed that the majority of the cells were nonadherent and that some cells exhibited veiled cell morphology (Fig 3A). Occasionally, a few colony-like clusters and adherent cells with typical dendritic cell and macrophage morphology were present, but in general, differentiation events in cultures containing FBS/RPMI were infrequent. The lack of differentiation events coupled with good viability (>85%), the presence of numerous mitotic figures (Fig 2), and a growth response measured by $[^{3}H]$-thymidine uptake (mean, 2,000 cpm; n = 4) in these short-term blast cultures indicated that ORL47 produces autocrine growth factors that favor self-renewal over differentiation. Notwithstanding, even with fresh media supplementation, the viability and growth response of these cultures began to decrease after 5 days. Growth in pooled AB normal human serum (NHS)/RPMI was also tested and yielded similar results (data not shown).

Differentiation and proliferative events associated with cytokine treatment. Normally, DC components represent less than 1% of PB. Because of the enhanced presence of veiled cells and adherent DC in the leukemic FBS/RPMI cultures, we compared the developmental response of ORL47 leukemic blasts with cytokine combinations that have been shown by us and others to favor DC development. As soon as 24 hours after cytokine treatment, changes in proliferation and maturational responses were noted. With respect to morphologic changes, GM-CSF treatment alone increased the number of processes found on the veiled cells and provoked the development of a few nonadherent CFU-like clusters (Fig 3B). Although the combination of TNF with GM-CSF increased the number of CFU-like clusters (Fig 3C), these remained nonadherent, and few mature DC progeny were noted. With SCF + TNF + GM-CSF treatment, the number of nonadherent clusters was increased even further, but again without the development of mature DC progeny (Fig 3D). Interestingly, the addition of IL-6,
ORL47 leukemic blasts underwent distinct responses to cytokines that resulted in the multistep development of mature dendritic cell and monocyte-macrophage progeny. (A) Growth in FBS/RPMI, (B) GM-CSF, (C) TNF + GM-CSF, (D) TNF + GM-CSF + SCF, and (E) TNF + GM-CSF + SCF + IL-6 is shown. Although differentiation events were distinguishable in cultures treated with TNF + GM-CSF and TNF + GM-CSF + SCF, the development of mono-DC-CFU-like clusters and mature DC progeny was accomplished most effectively with TNF + GM-CSF + SCF + IL-6. Note the apparent emigration of DC from the CFU-like clusters. (F) Treatment of cultures with TNF + GM-CSF and polyclonal anti-TNF at 10 μg/mL inhibited differentiation of the leukemic blasts. (Original magnification × 50.)

which has been previously shown to induce the development of certain leukemic cells, resulted in the terminal differentiation of DCs. By 24 hours of TNF + GM-CSF + SCF + IL-6 treatment, numerous adherent CFU-like clusters were present in the cultures, along with noncolony-associated adherent DCs (Fig 3E). Morphologically, these CFU-like clusters were identical to the monocyte-DC-CFU derived from normal cord blood or adult PB (Fig 4). Treatment of similarly prepared mononuclear cells from normals yielded less than 1% DC progeny (data not shown).

In Table 1 and Fig 5, we compare the proliferative events associated with ORL47 leukemic blasts treated with cytokines with culture in FBS/RPMI alone. As represented in Table 1, the largest increases in absolute cell counts (2.6-fold) were induced by TNF + GM-CSF + SCF treatment. These were followed in declining order by TNF + GM-CSF
A typical monocyte-DC colony developing from normal (neonatal cord blood) CD34+ progenitors treated with TNF + GM-CSF + SCF. Colony growth was assessed in semisolid media (.85% methylcellulose/15% FBS/RPMI). Photomicrographs were taken after 8 days of incubation. (Original magnification × 50.)

+ SCF + IL-6 (2.4-fold), TNF + GM-CSF (2.2-fold), and GM-CSF (2.0-fold). Thymidine uptake analysis showed that increases in proliferation in TNF + GM-CSF + SCF cultures were statistically significant \(P = .028\); Fig 5). Quantitative analysis at 72 hours by phase microscopy also showed only three mono-DC-CFU-like clusters in FBS/RPMI cultures as compared with more than 300 mono-DC-CFU-like clusters in TNF + GM-CSF + SCF cultures.

Because conditioned medium (CM) from the leukemic cell line HL60 is enriched in hematopoietically active cytokines, we tested the development of ORL47 in the presence of HL60 CM. The absolute cell number was increased above FBS/RPMI cultures with HL60 CM (Table 1), but these events were not associated with DC development, as assessed by phase microscopy. The addition of IL-6 to HL60 CM also failed to induce DC development. Subsequent studies showed that neither HL60 nor U937 leukemic cell lines or fresh leukemic cells from another patient (ORL49) were induced to differentiate along the DC pathway with any combination of cytokines tested (data not shown).

Differential analysis related to DC development. To help ascertain the position of the ORL47 leukemia in the myeloid pathway, the proportion of cells which differentiate into PMN was determined. Under all the conditions examined, including treatment with GM-CSF alone, less than 1% PMN cells developed in the cultures (Table 2). This is in contrast with the treatment of normal CD34+ progenitor cells with GM-CSF, which produces ~30% PMN progeny. Moreover, unlike normal CD34+ cell cultures treated with TNF + GM-CSF and anti-TNF antibodies, the addition of

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cells/mL ( \times 10^4 )</th>
<th>% Viability</th>
<th>Response*</th>
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</thead>
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<tr>
<td>FBS</td>
<td>0.9</td>
<td>86</td>
<td>—</td>
</tr>
<tr>
<td>GM-CSF</td>
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</tr>
<tr>
<td>HL-60 CM</td>
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<td>1.8</td>
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The number of cells/mL in the ORL47 cultures was determined with a hemacytometer 24 hours after treatment. Viability was measured by Trypan blue exclusion.

* Growth response relative to FBS cultures.
† Anti-TNF and Anti-GM-CSF were added together with TNF + GM-CSF, as described in the text. HL-60 conditioned media was used at a final concentration of 50% in RPMI-1640 culture medium. Results were obtained from a single experiment.

Table 2. PMN Content in ORL47 Cultures

<table>
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<tr>
<th>Condition</th>
<th>FCS</th>
<th>GM-CSF</th>
<th>TNF + GM-CSF</th>
<th>TNF + GM-CSF + SCF</th>
<th>TNF + GM-CSF + IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>% PMN</td>
<td>11.0</td>
<td>8.5</td>
<td>9.7</td>
<td>8.0</td>
<td>10.0</td>
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PMN content was determined by Wright-stain analysis; an average of 300 cells per condition were analyzed. Of the PMNs present, the majority in all instances were neutrophils.
anti-TNF to TNF + GM-CSF leukaemic cultures (described below) did not produce a shift from mononuclear cell (MNC) to PMN progeny. These results indicate that the clonal DC-mono- 

cyte progenitor cell unit in ORL47 is at a post-CFU-GM stage.

Effects of anti-TNF and anti–GM-CSF antibodies on leukaemic DC development. As previously described by us for normal DC differentiation, antibodies to TNF and GM-CSF inhibited leucemic DC development. In these experiments anti-TNF or anti–GM-CSF was added together with TNF + GM-CSF at the onset of the culture period. As depicted in Fig 3F, anti-TNF greatly reduced colony growth. The absolute cell content was decreased by ~30% and ~45% for anti-TNF and anti–GM-CSF, respectively, as compared with cultures treated with TNF + GM-CSF (Table 1). Thymidine uptake analysis also showed that addition of either antibody decreased proliferation (data not shown). Other investigators have shown that similar antibody treatment blocks proliferative responses in different leukaemias, in support of our observations.

NSE and phagocytic activity associated with DC and monocyte-macrophage development from ORL47 leukaemic cells. Leukaemic blasts treated with TNF + GM-CSF + SCF + IL-6 were stained in situ for NSE and phagocytic activity. As expected, there was no NSE or phagocytic activity in either colony-associate or free DCs (Fig 6, A and B). In contrast, monocyte-macrophages developing simultaneously from mono-DC-CFU exhibited both NSE activity and the capacity to ingest Ig-coated magnetic beads. In situ immunofluorescence analysis also showed that while expressing class II MHC antigens on their surface, these DCs did not express monocyte-macrophage–associated CD14 antigens (data not shown). Unexpectedly, free and colony-associated monocyte-macrophages were also negative for CD14, suggesting a leukaemic defect involving the CD14 protein.

The capacity of ORL47 cells to induce a mixed leucocyte reaction (MLR). The MLR stimulatory potential of ORL47 leukaemic cells cultured with TNF + GM-CSF + SCF, TNF + GM-CSF + SCF + IL-6, or FBS/RPMI alone was explored (Fig 7). At stimulator to responder cell ratios of 0.2:1, cultures containing stimulator cells treated with either TNF + GM-CSF + SCF or TNF + GM-CSF + SCF + IL-6 exhibited an equal proliferative capacity that was approximately threefold greater than that exhibited by cultures containing FBS/RPMI alone. The capacity to produce a potent MLR in these cultures was retained even at stimulator:responder ratios as low as 0.06:1, which is consistent with a DC-mediated T-cell response. Because there were few mature DCs in TNF + GM-CSF + SCF cultures, these results indicate that cells found at intermediate stages of DC development are capable of inducing potent MLRs.

DISCUSSION

We have described a malignant counterpart of the mono-DC-CFU in an AML and the multistep induction of mature progeny from these blasts. Differentiation was under tight cytokine control, with both DCs and monocytes arising from the leukaemic cells. Although proliferative events and partial differentiation were achieved with TNF + GM-CSF and TNF + GM-CSF + SCF (Table 1, Figs 3 and 5), subsequent development of mono-DC-CFU and terminal maturation of DCs was dependent on the addition of IL-6 (Figs 3 and 6). These results are in contrast with those previously published for the development of DCs from normal CD34+ stem cells that require only synergy between TNF and GM-CSF to achieve a large yield of DC progeny.

Because we were able to maintain short-term growth of ORL47 cells in the absence of stromal elements and exogenous cytokines, and achieve in vitro differentiation with the specified cytokines, it appears that ORL47 represents a differentiation positive leukaemia that produces autocrine factors that favor self-renewal over differentiation. Because GM-CSF increased the growth response of leukaemic cells with few differentiation events (Table 1, Fig 3B) and has previously been described to promote self-renewal in most AML, it is possible that GM-CSF is also involved in the self-renewal of ORL47.

Morphologically, development of the mono-DC progenitor cells treated with TNF + GM-CSF + SCF + IL-6 into colonies containing DCs and monocytes (Figs 3E and 6) was identical to that previously reported for CD34+ stem cells treated with either TNF + GM-CSF (Fig 3) or TNF + GM-CSF + SCF (Santiago-Schwarz F, in preparation). However, with respect to temporal kinetics, the development of mono-DC colonies from leukaemic cells occurred much more rapidly than from normal CD34+ stem cells (24 hours vs 5 days), suggesting that additional maturation steps were required in the CD34+ progenitor cell compartment. Leukaemia-derived mature DCs exhibited phenotypic and functional features of normal stem cell-derived DCs including the lack of NSE and phagocytic activity (Fig 6), the lack of CD14 expression, the presence of class II MHC antigens (data not shown), and the capacity to induce a potent MLR (Fig 7). In contrast, normal differentiation was maintained only partially in the monocytic compartment, as assessed by the presence of NSE and phagocytic activity (Fig 6), and the absence of surface CD14 antigens (data not shown).

Although leukaemic blasts treated with TNF + GM-CSF + SCF did not undergo terminal maturation into DCs, these cultures exhibited a potent MLR stimulatory capacity, equal to that of cultures containing terminally differentiated DCs (TNF + GM-CSF + SCF + IL-6). This observation indicates that cells found at intermediate stages of DC differentiation are functionally competent. In support of this, Thomas et al have recently shown that cultured CD33+CD13+ DC precursors are effective stimulators of a MLR before they differentiate into mature DC.

The dependency of ORL47 on exogenous IL-6 may not represent a unique leukaemic event, but an example of the regulatory role of secondary cytokines in myelopoiesis. IL-6 has been shown to be an important secondary cytokine during normal GM-CSF–induced monocytopoiesis. Because mono-mφs arising during development produced IL-6, a developmentally active cytokine loop involving IL-6 was suggested in this report. In other reports, IL-6 was found to be a secondary cytokine stimulated by TNFα that supported the development of CFU from AML blasts. Some AML blasts categorized as FAB M1-M4 do not produce IL-
6, but express IL-6 receptors, indicating that these leukemias are susceptible to the effects of IL-6. Although we did not determine the expression of IL-6 receptors on ORL47, their expression would be expected because ORL47 exhibited an FAB M2 phenotype and responded to IL-6. Based on these data, we speculate that the dependency of ORL47 blasts on exogenous IL-6 reflects the lack of endogenous secondary IL-6 production by a population of cells (probably mono-mφs), and that this cytokine might be produced as a secondary cytokine during mono-DC development from normal CD34+ progenitor cells. Some investigators have proposed that IL-6 may sustain leukemic blast development by preventing cell death by apoptosis. Because IL-6 seemed to promote the release of DC from the colonies as well as DC adherence, it may be that the effect is also adhesion molecule associated.

The inability to produce PMN progeny with GM-CSF (Table 2), and the lack of a MNC to a PMN cell deviation with anti-TNF treatment indicate that the clonal component in ORL47 is at a distinct post-GM-CFU stage, specifically primed for DC and monocyte-macrophage development. A CD33+CD13+ DC precursor has recently been described in normal PB that may be the normal equivalent of ORL47. We have also described an analogous post-CFU-GM, mono-DC progenitor in cord blood that resembles ORL47 (Santiago-Schwarz F, in preparation). Our attempts to induce DC development from the HL60 (GM-CFU) and U937 (M-CFU) leukemic cell lines and from leukemic blasts isolated from a patient with AML failed, further supporting the concept that ORL47 is a distinct leukemic DC progenitor cell unit that is at the post-GM-CFU stage.

It might be argued that some of the differentiation events

Fig 6. In situ analysis of mono-DC-CFU-like clusters for NSE (A) and phagocytic activity (B) in leukemic cultures treated with TNF + GM-CSF + SCF + IL-6. As previously described for normal DCs, colony-associated and free DC progeny either weakly express or lack NSE activity and are incapable of ingesting IgG-coated beads. Monocytes developing simultaneously in the leukemic cultures exhibit both NSE and phagocytic activity. Note monocytes-macrophages that have ingested beads in B. Cells stained for NSE were counterstained with hematoxylin. (Original magnification × 50.)
we describe are caused by the development of normal, and not leukemic progenitors. Whereas it is possible that nonleukemic progenitors are initially contained in our leukemic blast cell–enriched fractions, there are several arguments against their development. Foremost, normal progenitor cells die quickly (within 72 hours) in culture if not provided with the appropriate exogenous cytokines, and thus, would not have survived the 3- to 5-day preincubation period in FBS/RPMI. If the leukemic blasts were producing hematopoietically active cytokines such as GM-CSF, and normal progenitors were present, differentiation events should have occurred in FBS/RPMI cultures. Instead, differentiation events were virtually absent in these cultures. Finally, because PMN development would have been supported by GM-CSF, the lack of PMN progeny also argues against the presence of normal progenitors such as GM-CFU and GEMM-CFU.

In addition to lending further insight into the classification of leukemias and the DC pathway, these observations help clarify the relationship between LCH abnormalities and malignancies. Although there is a growing number of reports describing associations between AML and LCH, the origin of the LC component has remained undefined. It has been suggested that the histiocytic element in LCH arises from three ontogenically different routes, (GM-CFU, M-CFU, and mono-DC-CFU), it is likely that aberrations in the monocytic pathway are a more common form of AML. Some investigators have argued that monocytic leukemias may be precursors of LC. Other investigators have described an unusual AML in which DC-like elements developed from AML cells cultured in T-cell–conditioned medium. Like ORL47, this AML was unusually aggressive. Although the progenitor and specific cytokine growth requirements were not identified, the phenotypic and functional features of progeny derived from this leukemia were similar to ORL47 and indicate that it may be its equivalent.

By using an in vitro approach similar to the one used in this study, other leukemias can be easily screened for DC development. This type of developmental analysis could provide a basis for accurately including myelodendritic leukemias in leukemia classification schemes. Because of the strict requirements for achieving DC differentiation, any clinical strategy aimed at targeting such leukemias should consider the specific growth requirements of the DC pathway.

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Identification of a malignant counterpart of the monocyte-dendritic cell progenitor in an acute myeloid leukemia

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