Acute Leukemia Associated With the t(4;11) Chromosome Rearrangement: Ultrastructural and Immunologic Characteristics

By Janet L. Parkin, Diane C. Arthur, Candice S. Abramson, Robert W. McKenna, John H. Kersey, Richard L. Heideman, and Richard D. Brunning

The acute leukemia associated with the t(4;11) chromosome rearrangement is characterized by relatively consistent clinical features: occurrence primarily in young individuals, hyperleukocytosis, and poor response to therapy. This study describes the morphological, ultrastructural, and immunologic characteristics of the leukemic cells from ten patients with this type of leukemia. The morphological features of the leukemic blasts vary from lymphoid-appearing to monocytic. Ultrastructurally and cytochemically, some of the lymphoid-appearing blasts possess features of myeloid origin. The immunologic phenotype is characteristically $\text{E}^{-}$ $\text{Slg}^{-}$ $\text{CALLA}^{-}$ $\text{BA-1}^{-}$ $\text{BA-2}^{-}$ $\text{HLA-DR}^{-}$ and $\text{TdT}^{-}$. These findings suggest that the t(4;11)-associated acute leukemia represents a proliferation of an early myeloid progenitor cell.

THE ACUTE LEUKEMIA associated with the t(4;11) chromosome rearrangement has been described as a subtype of acute lymphoblastic leukemia, which has a poor prognosis when treated with conventional chemotherapy. The total number of cases of leukemia associated with this abnormality has been small, and detailed immunologic and morphologic studies have not been reported. Eight cases studied for E-rosettes and surface immunoglobulin were reported as non-T, non-B acute lymphoblastic leukemia.2,6

The present study describes the morphological, ultrastructural, ultracytochemical, and immunologic characterization of the leukemic cells from ten patients with the t(4;11) chromosome rearrangement. The clinical and cytogenetic features of six of these patients (cases 1, 3, 4, 5, 9, and 10) have been previously reported.6

MATERIALS AND METHODS

Eight of ten patients in this study were diagnosed at the University of Minnesota Hospitals between January 1978 and December 1981. Two patients were diagnosed at Denver Children's Hospital; one of these patients was referred to the University of Minnesota for treatment, and frozen cells from the other patient were studied for immunologic and ultrastructural characteristics at the University of Minnesota. Initial diagnostic blood and bone marrow specimens from all patients were studied by light microscopy and cytochemistry.

Cytogenetics

Cytogenetic studies were performed at diagnosis in eight of ten cases; seven at the University of Minnesota Hospital and one at Denver Children's Hospital. Two cases were studied at the University of Minnesota Hospitals at first relapse. Chromosome analysis was performed using direct preparations and short-term culture according to methods previously described.6

Light Microscopy and Cytochemistry

Blood and bone marrow smears were routinely stained with a Wright-Giemsa technique. Cytochemical procedures performed included myeloperoxidase (MPO), Sudan black B (SBB), nonspecific esterase (NSE), acid phosphatase (AcP), and periodic acid-Schiff (PAS).

Ultrastructural Studies

Electron microscopic studies were performed on fresh bone marrow cells from eight patients and on frozen bone marrow cells from two patients. The frozen cells had been stored for 1–2 yr. Specimens were processed by methods previously described.6 Except for patient 9, the specimens from all patients were obtained prior to chemotherapy.

Ultrastructural Cytochemistry

Ultracytochemical studies were performed on five cases; fresh blood and bone marrow cells in two and frozen bone marrow cells in three. Ultrastructural localization of peroxidase (MPO and PPO) was performed on these five cases using the methods of Graham and Karnovsky10 and Breton-Gorius et al.24 Cells were fixed for 1 hr at 4°C and then incubated at room temperature (RT) in a buffered diaminobenzidine/hydrogen peroxide solution for 1 hr. Control cells were incubated in a solution lacking the hydrogen peroxide.

Ultrastructural localization of nonspecific esterase (NSE) was performed on four cases using a modification of the method of Payne et al.11 Cells were fixed for 1 hr in 0.1 M phosphate-buffered 2.5% glutaraldehyde with 2% paraformaldehyde added (pH 7.4), and then stored in phosphate buffer for at least 4 hr at 4°C. After rinsing in buffer, cells were incubated at RT for 2 hr in a solution containing 2-naphthylthiol acetate (NTA) as the substrate and Fast Blue BB salt as the coupling agent, followed by postfixation in 1% buffered osmium tetroxide. Control specimens were incubated with elimination of either the NTA or the osmium tetroxide.

Serum Lysozyme

Serum lysozyme levels were determined in four of eight cases using the method of Perry and associates.12

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Submitted February 25, 1982; accepted July 13, 1982.

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0006-4971/82/6006-00/4 $02.00/0
Membrane Surface Markers

Immunologic marker studies were performed on blood and/or bone marrow cells from nine of the ten patients. All cell samples utilized contained greater than 80% malignant cells. The specimens were depleted of red blood cells by lysis with Tris-NH4Cl according to the method of Boyle. Cells were then centrifuged, washed twice in RPMI 1640 medium, and either examined immediately or suspended in 10% DMSO for cryopreservation in liquid nitrogen.

Surface Immunoglobulin (SIg). Cytoplasmic Immunoglobulin (CIg). and “Sheep” Erythrocyte Rosette (E-Rosette) Studies

SIg and CIg staining were performed according to methods previously described. E-rosettes were prepared by the method of Kaplan using AET-treated red blood cells.

Monoclonal Antibody Testing

The monoclonal antibodies BA-1, BA-2 (anti-p24), J-5 and BA-3 (anti-CALLA), and 7.2 (anti-HLA-DR) have been previously described. Indirect immunofluorescence was used to assess binding of monoclonal antibodies to various cell populations. Target cells were washed and resuspended to 1.5 x 10^7/ml in phosphate-buffered saline (PBS, pH 7.2) containing 2.5% FCS and 0.02% sodium azide (BPFS buffer). Appropriately diluted monoclonal antibody or control ascitic fluid was then mixed with an equal volume of target cells and incubated for 40 min on ice. After washing two times in cold BPFS buffer, cells were stained with fluorescein isothiocyanate (FITC) goat anti-mouse Ig (F/P ratio = 3.7 mg/g, Meloy Laboratories, Inc.) at a dilution of 1:16. Incubation was continued for 40 min on ice and the cells were then washed 3 times in BPFS buffer and mounted under glass cover slips. Examination of cells was done with a Zeiss fluorescent microscope equipped with Ploem epi-illumination. Populations were scored as negative if less than 10% of cells were positive, weakly positive if 11%–50% of cells were positive, and strongly positive if greater than 50% of cells were positive in fluorescence.

Terminal deoxynucleotidyl transferase (TdT) activity was determined on seven cases with an immunofluorescence technique using antibody obtained from Bethesda Research Laboratories, Gaithersburg, Md. Fresh bone marrow cells were studied in five cases, frozen bone marrow cells in one, and frozen blood cells in one. The TdT was determined in one case by biochemical assay.

RESULTS

Incidence

All seven patients in this study who had G-banded bone marrow chromosomes analyzed at the University of Minnesota Hospitals at the time of diagnosis (cases 1, 3, 4, 5, 6, 8, and 10) had a modal number of 46 chromosomes with the t(4;11) (q21; q23) as the sole karyotypic abnormality (Fig. 1). Since October 1973, banded marrow chromosomes have been successfully analyzed in a total of 77 patients with acute lymphoblastic leukemia at the time of diagnosis at this institution (44 children and 33 adults). Thus, we have found the t(4;11) in 11% of children and 7% of adults. These figures may be spuriously high because these high-risk patients with marked leukocytosis are more likely to be referred to the University of Minnesota for evaluation than are low-risk patients.

Clinical and Laboratory Data

The clinical, laboratory, and cytogenetic data are summarized in Table 1. The patients ranged in age from 8 wk to 33 yr. Seven were younger than 16 yr; 3 were 3 mo of age or less. The leukocyte counts ranged from 34 x 10^9/liter to 770 x 10^9/liter; the median was 450 x 10^9/liter. Hemoglobin at diagnosis ranged from 4.0 to 10.9 g/dl, with a median of 8.0 g/dl. The platelet counts ranged from 6 x 10^9/µl to 165 x 10^9/µl; the median was 55 x 10^9/µl. Splenomegaly was a feature common to most patients; the spleen tips ranged from 2 to 9 cm below the left costal margin. There were no patients with mediastinal masses or lymphadenopathy greater than 3 cm. Serum lysozyme was elevated to 29 in one patient (case 9).

Therapy

Eight of ten patients achieved an initial remission with standard induction therapy for acute lymphoblastic leukemia (ALL) including vincristine (Vcr), prednisone (Pdn), and l-asparaginase. One of these eight had failed to achieve remission after two courses of adriamycin and cytosine arabinoside. One patient failed to achieve remission with the above ALL regimen but was successfully induced with Vcr, Pdn,


Table 1. Clinical Characteristics at Diagnosis, Response to Therapy, and Cytogenetic Studies

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>WBC (x 10^9/Liter)</th>
<th>Survival (mo)</th>
<th>Time of Study</th>
<th>Number of Cells Examined</th>
<th>Banded Karyotypes</th>
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<td></td>
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<td>Normal Clonal</td>
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<td>Remission</td>
<td>46, XX, t(4;11)(q21;q23)</td>
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<tr>
<td>1</td>
<td>16 mo</td>
<td>F</td>
<td>150</td>
<td>11</td>
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<td>46, XX</td>
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</tr>
<tr>
<td>2</td>
<td>7 yr</td>
<td>M</td>
<td>340</td>
<td>15</td>
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<td>16</td>
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<td>5</td>
<td>15 yr</td>
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<td>572</td>
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<td>Clone 2: 46, XY, t(4;11)(q21;q23), q(15)</td>
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<tr>
<td>6</td>
<td>33 yr</td>
<td>F</td>
<td>600</td>
<td>4</td>
<td>Diagnosis</td>
<td>11, 1, 10</td>
<td>46, XX/46, XX, t(4;11)(q21;q23)</td>
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<td></td>
<td>Remission</td>
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<td>46, XX</td>
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<tr>
<td>7</td>
<td>8 wk</td>
<td>M</td>
<td>275</td>
<td>15</td>
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<td>46, XY</td>
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<td>Relapse</td>
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<td>50, XY, t(4;11)(q21;q23), + ? + 10, + 15, + 18, + 19</td>
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<td>8</td>
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<td>Diagnosis</td>
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<td>46, XX, t(4;11)(q21;q23), del(7)(q15)</td>
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<tr>
<td>9</td>
<td>31 yr</td>
<td>F</td>
<td>34.4</td>
<td>10</td>
<td>Relapse</td>
<td>18, 1, 17</td>
<td>46, XX/46, XX, t(4;11)(q21;q23), del(7)(q15)</td>
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<tr>
<td>10</td>
<td>8 wk</td>
<td>F</td>
<td>770</td>
<td>5</td>
<td>Diagnosis</td>
<td>3, 0, 3</td>
<td>46, XX, t(4;11)(q21;q23)</td>
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<td></td>
<td>Relapse</td>
<td>10, 0, 10</td>
<td>46, XX, t(4;11)(q21;q23), del(17)(q15)</td>
</tr>
</tbody>
</table>

Cyclophosphamide, and adriamycin. One patient responded to initial treatment with Vcr, Pdn, and adriamycin. All patients received central nervous system intensification with intrathecal methotrexate (MTX) and 1800 rad cranial irradiation. Maintenance medications included Vcr, Pdn, 6-mercaptopurine, and MTX in all patients, with monthly pulses of cytosine arabinoside, cyclophosphamide, and adriamycin in five patients. The duration of initial remission has been short, ranging from 1 to 13+ mo (median 3.5 mo). No sustained second remissions have been achieved with aggressive chemotherapy or allogeneic bone marrow transplantation (two patients). Only two patients are currently alive: one is in remission following an initial remission of 4 mo and one continues in initial remission at 13+ mo. Survival of the other eight patients ranged from 4 to 16 mo (median 10.5 mo).

Morphology and Cytochemistry
In eight of the ten patients the predominant leukemic cells were lymphoid in appearance; there were minor populations of blasts with relatively abundant blue cytoplasm and occasional blasts with coarse azurophilic granules. Several of these eight cases were characterized by a population of blasts that were small with markedly hyperchromatic nuclei (Fig. 2).

In two of the ten patients the blood and bone marrow smears were characterized by a dimorphic blast population with approximately equal numbers of monocytic and lymphoid-appearing blasts (Fig. 3). In one of these two patients, rare neutrophils with pseudo-Pelger-Huet nuclei were noted. Following unsuccessful chemotherapy, the blast population in the latter patient was exclusively monoblastic (Fig. 4).

The myeloperoxidase stain was performed in all ten cases; in nine the blasts were MPO negative. In one of the cases with a predominantly lymphoid-appearing blast population, occasional MPO-positive lymphoid blasts were present in the blood and bone marrow smears. Of the blasts in all patients, 10%–90% were PAS positive (Fig. 2). The reaction was in the form of granules and globules, and in many cases, a high percentage of the blasts was intensely positive. Blasts from three cases with predominately lymphoid mor-
Fig. 2. (A) Peripheral blood smear from case 6. The blast population is predominantly lymphoid in appearance. Occasional lymphoid-appearing blasts in this case were peroxidase positive by light microscopy and approximately 10% manifested peroxidase positivity with ultracytochemistry (see Fig. 8). Occasional Auer rods were noted on ultrastructural examination (see Fig. 6) (Wright-Giemsa, x400). (B) Peripheral blood smear reacted with periodic acid-Schiff. The blasts contain numerous positive globules and granules (periodic acid-Schiff, x400).

Fig. 3. (A) Initial bone marrow smear from case 6 illustrating the mixed monoblastic and lymphoid population (Wright-Giemsa, x400). (B) Nonspecific esterase reaction on a smear from the same case illustrating several positively reacting monocytoid blasts. The lymphoid cells are negative (nonspecific esterase, x400).

Fig. 4. Bone marrow smear from case 7 at time of relapse illustrating an exclusively monoblastic population of leukemic cells (Wright-Giemsa, x400).

phology manifested scattered focal deposits of nonspecific esterase activity. In one patient (case 3), a majority of the blasts exhibited moderately intense, focal paranuclear NSE activity. In the two morphologically dimorphic cases, the monocytoid population of blasts manifested diffuse NSE staining (Fig. 3); the lymphoid-appearing blasts were nonspecific esterase negative. Several of the lymphoid-appearing blasts in these two patients also contained coarse azurophilic granules that were myeloperoxidase negative. Eight of nine cases studied manifested weak to moderate scattered granular acid phosphatase positivity in most of the leukemic blasts.

In four patients (cases 6, 8, 9, and 10), the classification of the leukemic process was problematic. Two (cases 8 and 9) presented with a dimorphic pattern with populations of monocytoid blasts and lymphoid-appearing blasts. The lymphoid-appearing blasts were marked by fairly intense blocks and granules of PAS positivity. On relapse, the blast population in one patient (case 9) consisted primarily of monoblasts that were slightly to intensely NSE positive and there was an elevated serum lysozyme level. The karyotype of 17
of 18 cells studied at this time showed a t(4;11) translocation (Table 1). In the other patient, the relapse was characterized by a monomorphous lymphoid-appearing population. In a third patient, the initial marrow smear contained approximately 5% erythroblasts, many of which manifested nuclear abnormalities; the periodic acid-Schiff reaction was positive in many of the erythroid precursors. Although the major blast population had lymphoid characteristics, the red cell findings suggested the possibility of a myeloid leukemia with some red cell differentiation. In the fourth patient (case 6), a low percentage of the lymphoid-appearing blasts manifested MPO and SBB positivity.

Ultrastructure

Ultrastructural and ultracytochemical studies of the ten cases are summarized in Table 2. The patients were divided into two groups on the basis of the ultrastructural characteristics of the blast population. In group I, comprising eight cases, the predominant blast population appeared lymphoid. In five of these eight cases (cases 3–7), from 10% to 50% of the lymphoid-appearing blasts contained large variably electron-dense structures that resembled abnormal mast cell/basophil granules (Fig. 5). Dysplastic myeloid cells, including micromegakaryocytes, hypogranular neutrophils and giant neutrophil precursors, were found in three cases (3, 5, and 6). Rare neutrophil myelocytes containing Auer rods were found in one case (case 6) (Fig. 6).

A minor population of monocyteoid blasts was present in two cases from group I (2 and 5); these blasts were larger than the lymphoid-appearing blasts and contained nuclei that were indented and contorted and had a delicate chromatin pattern. The cytoplasm was abundant and contained numerous small electron-dense haloed granules, rounded profiles of rough endoplasmic reticulum and small bundles of microfibrils (Fig. 7). A minor population of erythroid-appearing

<table>
<thead>
<tr>
<th>Table 2. Summary of Ultrastructural, Ultracytochemical, and Immunologic Characteristics (10 Cases)</th>
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<tbody>
<tr>
<td>Patient</td>
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*Frozen cells.
†Posttherapy specimen.
‡Biochemical determination (normal range 0–35).
ND, not done; BM, bone marrow; PB, peripheral blood; MPO, myeloperoxidase; PPO, platelet peroxidase; NSE, nonspecific esterase.
blasts present in one case (case 8) was characterized by cells with abundant cytoplasm that contained numerous polyribosomes and an active golgi zone with clusters of small electron-dense granules.

Ultracytochemistry was performed on the blasts from four patients in this lymphoid group and the results are summarized in Table 2. Of particular note were the findings in cases 6 and 8. In case 6, approximately 10% of the lymphoid-appearing blasts contained granules with either myeloperoxidase or platelet peroxidase activity (Fig. 8). There was no peroxidase activity in the perinuclear envelope or rough endoplasmic reticulum. This case was also characterized ultrastructurally by the presence of rare neutrophil myelocytes with Auer rods. In contrast to the 5%–10% of blasts with peroxidase-containing granules on ultracytochemistry, only occasional blasts with MPO activity were identified by light microscopy.

In case 8, which was characterized by abnormal-appearing erythroid precursors on light microscopy, approximately 5% of the blasts showed diffuse cytoplasmic peroxidase positivity associated with ribosomes characteristic of the pseudoperoxidase positivity of hemoglobin.

In group II, consisting of two cases (9 and 10), the ultrastructural studies confirmed the monocytoid differentiation evidenced in light microscopy (Fig. 9). In case 9, all of the blasts were large cells with monocytoid nuclei, very large nucleoli, and abundant cytoplasm that contained numerous small haloed electron-dense granules, scattered polyribosomes, and small perinuclear bundles of microfilaments. The blasts were irregular in outline and showed thin cytoplasmic projections. In case 10, approximately 80% of the leukemic cells were monocytic and 20% were lymphoid in appearance. Approximately 50% of the monocytic blasts were intensely NSE positive (Fig. 10); the NSE was localized to the small haloed granules and to large membrane-bound structures that appeared to represent cisterna of smooth endoplasmic reticulum. Rare perinuclear NSE activity was also noted. The percentage of NSE-positive granules was variable and ranged...
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from approximately 20% to 100% of the granules in different blasts. Rare lymphoid blasts showed NSE positivity in the form of large granules, some of which were located inside cytoplasmic vacuoles. A limiting membrane could not be clearly distinguished around most of these large NSE-positive structures.

Immunologic Markers

Immunologic marker data from nine of the ten patients are summarized in Table 2. The leukemic cells from eight of nine patients studied for membrane surface markers were negative for E-rosette, surface immunoglobulin, and common acute lymphocytic leukemia associated antigen (CALLA); the blasts from one patient were CALLA positive. Low numbers of cytoplasmic immunoglobulin-positive cells were observed in three patients. The cells from all eight patients tested gave a positive reaction with the anti-p24 antibody, BA-2, and cells from seven of nine patients reached strongly with anti-HLA-DR. The antigen detected with antibody BA-1 was either absent (five patients) or weakly expressed (three patients) in eight cases tested. TdT-positive cells were observed in seven of eight specimens tested; the percent of positive cells was 15%, 15%, 29%, 30%, 42%, 49%, and 80%. The negative result in one case was obtained by biochemical assay and occurred in the only case that was CALLA positive.

DISCUSSION

The acute leukemia associated with the t(4;11) chromosome abnormality has been generally characterized by clinical and laboratory features that have
Fig. 7. Bone marrow monocytophoid blast from case 8 characterized by an indented nucleus, large nucleolus, small bundles of microfilaments, and scattered small electron dense granules (uranyl acetate, lead citrate, ×13,000).

distinguished it from the more common type of acute lymphocytic leukemia. The patients with this form of leukemia usually have marked leukocytosis, splenomegaly, and an aggressive clinical course. Several cases have occurred in very young individuals and four have been classified as congenital leukemia.

This leukemia appears to be identified with unusual morphological and ultrastructural features characterized by mixed monocytic and lymphoid cell populations and lymphoid-appearing blasts that ultrastructurally and cytochemically may manifest myeloid markers. Some of these findings, i.e., evidence of basophil and/or mast cell differentiation, are similar to the observations in some cases of blast transformation of chronic myeloid leukemia (CML) or Philadelphia chromosome (Ph1) positive acute lymphocytic leukemia. Also similar to "lymphoblastic" crisis of CML, the blasts in t(4;11)-associated acute leukemia may manifest TdT positivity. Although the lymphoid-appearing blasts in Ph1-positive lymphoblastic leukemia are frequently CALLA positive, the blasts in t(4;11)-associated acute leukemia in this study were, with one exception, CALLA negative.

The seemingly contradictory morphological, ultrastructural, and cytochemical data in some of the present cases of t(4;11)-associated acute leukemia could be explained by a proliferative process involving an early myeloid precursor cell that has a lymphoid appearance in light microscopy. Breton-Gorius and colleagues have demonstrated that the lymphoid-appearing blasts in CML lymphoblastic crisis may in
some instances manifest ultracytochemical evidence of megakaryoblastic differentiation. The presence of the basophil/mast cell granules that has also been observed in the lymphoid blasts in CML in blast crisis is further supportive evidence of myeloid participation in the leukemic process.

Alternatively, the t(4;11)-associated leukemia could involve a progenitor cell with the potential to differentiate along both myeloid and lymphoid lines, i.e., a mixed leukemia. This, however, would not appear to be an adequate explanation for the ultrastructural and ultracytochemical demonstration of myeloperoxidase, platelet peroxidase and mast cell/basophil granules in some of the lymphoid-appearing blasts.

Immunologic marker studies in the present group of patients resulted in a rather consistent cell phenotype: E− Slg− CALLA− BA-1− p24(BA-2)+ HLA-DR+.

This phenotype contrasts with the majority of cases of ALL, which have the following phenotype: E− Slg+ CALLA− BA-1− p24(BA-2)+ HLA-DR+. The immunologic data in these patients are consistent with the hypothesis that the majority of cells in patients with t(4;11) leukemia are nonlymphoid in nature.

These studies identify the acute leukemia associated with the t(4;11) chromosome rearrangement as a proliferation of cells that may manifest characteristics that have been associated with both myeloid and lymphoid cells. These findings suggest the possibility that the proliferative process involves a bone marrow precursor cell that has the capacity to differentiate towards both myeloid and lymphoid cells or that the myeloid progenitor cell may at some stage of development have morphological and biochemical features that are commonly attributed to lymphoid cells.
REFERENCES


Fig. 9. A group of bone marrow monocytoid blasts from case 10 showing typical monocyte features, including small cytoplasmic granules and irregular cell borders (×5000, uranyl acetate, lead citrate).
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