Functional and Morphologic Characteristics of the Leukemic Cells of a Patient With Acute Monocytic Leukemia: Correlation With Clinical Features

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The clinical course of a patient with acute monocytic leukemia and prominent infiltration of the skin and testes is described. In vitro studies demonstrated that the circulating monocyte precursors were capable of adherence to nylon fibers, and phagocytosis of bacteria and latex particles. In vivo, migration of leukemic cells to skin windows was observed. Extreme nuclear folding, marked surface activity, and morphologic features suggesting nuclear and cytoplasmic maturation were seen by light and electron microscopy. The presence of morphologically and functionally more differentiated monocytic cells may account for the marked tissue invasion in this patient and, possibly, in other patients with monocytic leukemia.

Organ infiltration by malignant cells is not uncommon in patients with leukemia, especially in the later stages of the disease. It has been recognized for many years that patients with monocytic leukemia are particularly prone to extramedullary involvement, and many cases have been described in which the disease presented with dermatologic manifestations. It is unusual, however, for such extramedullary involvement to dominate the clinical course of patients treated with modern chemotherapeutic techniques. This report describes a patient in whom testicular and skin infiltration reached massive proportions despite vigorous therapy and correlates these clinical manifestations with certain in vivo and in vitro characteristics of the patient's leukemic cells.

CASE REPORT

JM was a 58-yr-old, white commercial fisherman who presented in April 1973 with a 2-wk history of severe headache and malaise. The patient had also noted the development of subcutaneous nodules on his elbows and forehead which had increased in size in the 3 wk prior to admission. Physical examination revealed subcutaneous nodules near each elbow, on the left foot, and on the forehead. Bilateral axillary adenopathy and hepatosplenomegaly were also present. The testes and gingiva were normal. On admission the hematocrit was 25%, platelet count was 170,000/μl, and the white blood cell count was 21,600/μl. Differential white cell count revealed 73% immature monocytic cells, 8% lymphocytes, 13% neutrophils, and 6% band forms. The majority of the immature monocytic cells showed some degree of maturation in that the cytoplasm was either lightly basophilic or slightly eosinophilic with minimal cytoplasmic granulation. Prominent nuclear folding was present in these cells. In addition, there was a population of blasts which were char-

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characterized morphologically by deeply basophilic cytoplasm, a higher nuclear/cytoplasmic ratio, and less prominent nuclear folding.

A bone marrow aspirate was hypercellular, and 70% of the cells present were monoblasts (Fig. 1). A higher percentage of the cells in the bone marrow than in the peripheral blood were frank blasts. Almost all of the circulating and marrow monocytic cells were peroxidase negative. Megakaryocytes which had phagocytized neutrophils were also observed. Serum muramidase was 115 μg/ml (normal, 10-20 μg/ml). Of note was a lumbar puncture which showed normal pressure, protein of 52 mg/100 ml, and glucose of 63. Cell count revealed 42 lymphocytes and 2 monocytes per μl. Many monoblasts were noted on a cytocentrifuge preparation. The cerebrospinal fluid cleared after induction therapy alone and remained normal throughout the patient's entire course.

The patient received induction therapy with daunorubicin, and complete remission was achieved with one course of chemotherapy. Biopsy of the subcutaneous nodules, performed several days after induction therapy was initiated, revealed nonspecific inflammation without evidence of leukemia cutis. After remission was achieved, the patient received consolidation and maintenance therapy with daunorubicin, cyclophosphamide, and guanazol.

In August 1973, prior to a course of maintenance therapy, the patient presented with enlargement of the left testis. The left testis was firm, nontender, and 2 cm larger than the right testis. A diagnosis of epididymitis was made by a consultant urologist, and only local therapy was administered. A bone marrow aspirate was normal, and the patient received further maintenance chemotherapy. The testis returned to normal size during the next 2 wk but enlarged once more in the subsequent 2 wk. A marrow aspirate at this time revealed 30% monoblasts. A needle aspirate of the left testis was performed and revealed extensive infiltration with immature monocytic cells. In retrospect, it was felt that the leukemic testicular infiltrate had preceded the bone marrow relapse by 1 mo, and had temporarily responded to maintenance chemotherapy.

The patient was re-induced with daunorubicin and received 60Co Cobalt, 600 R, to the left testis, resulting in reduction to near-normal size. A partial bone marrow remission was achieved with daunorubicin, but 4 wk after chemotherapy, testicular swelling and reappearance of erythematous subcutaneous nodules occurred. Bone marrow aspirate revealed florid relapse. Biopsy of the skin...
nodules revealed a malignant subepidermal infiltrate consistent with leukemia cutis. Leukemia cutis became generalized 2 wk after its onset and became a tremendous burden to the patient both physically and emotionally (Fig. 2). The patient admitted to avoiding personal contact with the staff and other patients because of extreme self-consciousness related to his disfigurement. Spot surface irradiation of single doses of 250-400 R to selected skin lesions produced only minimal reduction in size. Further chemotherapy with guanazole, followed by azacytidine, was administered. Although partial bone marrow, skin, and testicular responses were observed with each course of therapy, another complete remission was never obtained.

By March 1974, there was marked physical deterioration. An episode of septicemia occurred while the patient was granulocytopenic, and the patient expired 11 mo after therapy for leukemia was initiated. An autopsy was performed and revealed bilateral lower lobe pneumonia and leukemic involvement of the skin, liver, spleen, pancreas, left and right testes, and possibly the anus.

MATERIALS AND METHODS

Skin windows were performed using the technique of Rebuck and Crowley.5 The initial cover slip was removed after 4 hr and was replaced with another cover slip which remained in place an additional 14 hr. Both were then stained with Wright's stain. Skin windows were performed on admission, before chemotherapy was initiated, and again during relapse at a time when the bone marrow was acellular and the circulating white blood cell count was 100/μl. Multiple nodular skin lesions were still present at this time despite the marrow-suppressive effect of chemotherapy.

Phagocytosis and adherence studies were performed late in the patient's course, just prior to the administration of chemotherapy for relapse. The white blood cell count was 14,600/μl, with 73%, blasts, 10%, neutrophils, and 17% lymphocytes at this time. To assess the phagocytic capacity of the leukemic cells, 1 ml heparinized blood was incubated for 30 min at 37°C with gentle tumbling, with either 10 μl of latex particles (Bacto-Latex, Difco, Detroit, Mich.) or a laboratory strain of Staphylococcus aureus. In both cases this represented an excess of particles to circulating cells. Slides were prepared and stained with Wright's stain.

Adherence to nylon fibers was tested by passing 4 ml of whole heparinized blood by gravity through a tuberculin syringe filled with 200 mg of nylon fibers (Leukopak, Fenwal, Morton Grove, Ill.) to a volume of 1 ml. Differential counts were performed on pre- and postfilter samples, and the per cent adherence of immature monocytic cells was calculated. The experiment was performed at room temperature in duplicate. In this system, approximately 65%, of neutrophils from normal individuals adhere to the nylon fibers.6

Samples of bone marrow and peripheral blood obtained before treatment were processed for...
RESULTS

Skin Windows

At both 4 and 18 hr, the skin window performed on admission demonstrated migration of cells with folded nuclei and occasional nucleoli (Fig. 3) which closely resembled the circulating monocytic cells of intermediate maturity. Migration was maximal on the 18-hr cover slip. These cells had moderately basophilic cytoplasm. Less mature cells, characterized morphologically by deeply basophilic cytoplasm, a high nucleus/cytoplasm ratio, and less nuclear folding, did not migrate. No migration of cells was noted when the patient had a low circulating white blood cell count.

Phagocytosis

Ingestion of bacteria and latex particles was noted in 91% and 75%, of the circulating leukemic cells, respectively. Multiple organisms and particles were ingested by some cells. Morphologically less mature cells were able to phagocytize both latex and bacteria. Phagocytic vacuoles containing ingested bacteria were observed.

Adherence

Sixty-three per cent of the leukemic cells adhered to the nylon fibers. Seventy-five per cent of the neutrophils adhered, while there was no adherence by the patient’s lymphocytes. The latter observation suggested that active adherence by the leukemic cells, rather than nonspecific trapping in the spaces between the fibers, occurred.
Fig. 4. One-micron plastic embedded section of sedimented peripheral blood leukocytes stained with borated toluidine blue (× 1250). Note the bizarre pleomorphic nuclei of the leukemic cells. A few normal neutrophils are present.

**Micromorphology**

Specimens processed for electron microscopy and viewed with the light microscope showed large, 20-40-μm cells with complex, convoluted nuclei within abundant, moderately basophilic cytoplasm (Fig. 4).

Electron micrographs revealed strikingly pleomorphic, lobate, and vermiform nuclei (Fig. 5). The aberrant nuclear shapes and the many chromatinic bridges enclosing small areas of cytoplasm enormously increased the interface between nucleus and cytoplasm. Chromatin was finely divided except for sparse aggregates of heterochromatin marginalized along the nuclear membrane. The cytoplasm contained a few flattened cisternae of rough endoplasmic reticulum, inconspicuous elements of the Golgi complex, occasional bundles of microfilaments, and several scattered electron-dense, variably shaped small granules. Cytoplasmic vacuoles and microvillous processes were observed in all cells.

In preparations of pretreatment bone marrow and peripheral blood cells incubated with *E. coli*, exuberant responsiveness was demonstrated by considerable surface activity. Increased numbers of microvillous projections and loops formed by extrusions of the plasma membrane were seen. Although some ingestion of bacteria was observed, there was little evidence of bacteriolysis.

**DISCUSSION**

Cellular morphology and marked elevation of serum muramidase allowed the diagnosis of monocytic leukemia to be made in this patient on admission.
Significant organ involvement was probably present at that time, as evidenced by the presence of hepatosplenomegaly, adenopathy, skin lesions, and abnormal monocytic cells on examination of the cerebrospinal fluid. All of these abnormalities disappeared when remission of leukemia was achieved.

On rare occasions in adults it is difficult to distinguish clinically and morphologically between acute monocytic leukemia and the leukemic phase of diffuse histiocytic lymphoma (DHL), particularly when organ or lymph node infiltr-
tion is present at the time of diagnosis of leukemia.\textsuperscript{9} In two recent reports emphasizing morphologic, functional, and cytochemical criteria, it was suggested that monocytic leukemia and DHL represent variants of similar disorders differing in the degree of development along the promonocyte-monocyte-tissue histiocyte line.\textsuperscript{10,11} The circulating blasts in a patient with DHL in a leukemic phase, described by Catovsky and Galton, were strongly muramidase positive, indicating a monoblastic rather than a lymphoblastic or myeloblastic origin of these cells.\textsuperscript{12} Elevation of serum muramidase is not a feature of DHL,\textsuperscript{13} however, as muramidase content tends to decrease with further maturation towards a fixed tissue macrophage.\textsuperscript{14} In contrast, Lukes and Collins, utilizing newer techniques of T- and B-cell surface markers and morphologic studies of lymph node histology, have concluded that the malignant cell in DHL is usually of lymphocytic rather than histiocytic origin.\textsuperscript{15} In terms of therapy, the classification proposed by Lukes and Collins is consistent with the responsiveness observed in DHL to agents (vinca alkaloids, alkylating agents, prednisone) effective in lymphocytic lymphoma. It has been reported by Shaw, however, that monocytic leukemia can be resistant to antileukemic agents and responsive to antilymphoma agents.\textsuperscript{11} Our experience is somewhat different in that we have observed complete remissions in many patients with acute monocytic leukemia utilizing daunorubicin and cytosine arabinoside alone or in combination. In patient JM, a complete remission was achieved after a single course of therapy with daunorubicin. To our knowledge, functional studies have not been performed using circulating blasts from patients with DHL. Additional therapeutic, morphologic, and immunologic studies are clearly needed to clarify the relationship, if any, between the monocytic leukemias and the lymphomas.

Testicular enlargement, which almost certainly was due to infiltration by leukemic cells, preceded marrow relapse of leukemia by 1 mo. This clinical impression was subsequently confirmed by biopsy of the testes. We have recently treated two additional patients with myelomonocytic leukemia in whom leukemic infiltrates of the skin and testes were the first and only signs of relapse of leukemia. On the basis of this experience, it is recommended that all suspicious lesions be biopsied so that appropriate local and/or systemic therapy for leukemia can be administered. Testicular involvement responded partially to local radiotherapy and chemotherapy but persisted until the patient’s death.

Considerably more distressing to the patient were the multiple large disfiguring leukemic skin nodules which developed during the first relapse. These lesions were moderately tender and pruritic, and because of their prominence on the face produced a great deal of emotional anguish and embarrassment. Although partial regression of the skin lesions occurred with chemotherapy and radiotherapy to the largest lesions, the nodules never totally disappeared, even when partial bone marrow remission was achieved with guanazole therapy.

Electron microscopy demonstrated an unusual degree of nuclear convolution and surface activity not readily appreciated by light microscopy. Slight condensation and margination of heterochromatin and the relative absence of nucleoli were indicative of nuclear maturation, while the small number of peroxidase-negative cytoplasmic granules was suggestive of some cytoplasmic maturation.\textsuperscript{10} It should be emphasized that these cells were morphologically
quite bizarre and did not conform to generally accepted criteria for normal
monocytes or their precursors. Rather, they closely resembled the malignant
monocytes described by Freeman and Journey in a report on the ultrastruc-
tural features of monocytic leukemia. As in our patient, irregular, deeply in-
dented nuclei with dispersed chromatin, perinuclear filaments, nuclear bridging,
small cytoplasmic granules, and pseudopodal cytoplasmic extensions were
present in the cases described by these investigators. Recently, Glick and
Horn also reported similar ultrastructural findings in several patients with
acute monocytic leukemia.

Skin window experiments demonstrated an intact and perhaps enhanced
ability of the circulating monocytoid cells to migrate to a cutaneous inflam-
matory site. The electron-microscopic demonstration of increased surface
membrane activity and microvillous formation in response to a phagocytic
stimulus is consistent with this in vivo observation. This may, in part, explain
the subsequent clinical course. Presumably, cells capable of further division
which migrated into the tissues were either protected from or were nonrespon-
sive to chemotherapy and survived and multiplied until they became clinically
apparent. Evidence that proliferation occurred in situ, as opposed to continued
reseeding, was provided by the negative skin window study at the time when the
patient had active, enlarging skin nodules but no circulating leukocytes.
Schmalzl et al. have shown that monoblasts are capable of migration and nor-
mal differentiation into macrophages on skin window cover slips and concluded
that leukemic monocytes share some functions with normal monocytes. These
investigators, as well as Ohta and Matsuda, noted that monoblast migration
tended to occur on the early cover slips and regarded this as a possible specific
feature of monocytic leukemia. Although some monocyte migration occurred
as early as 4 hr in our patient, migration was far greater on the later cover slip.
Our observation is, therefore, at variance with these other reports. Ohta and
Matsuda also detected a rapid diminution over time in muramidase staining of
monocytes which had migrated to cover slips and postulated that it was this
ready release of the enzyme which resulted in the serum elevation seen in pa-
tients with monocytic leukemia.

We have recently treated another patient with typical monocytic leukemia
whose circulating blasts demonstrated phagocytic competence and migration to
skin windows comparable to that seen in patient JM. As in patient JM, func-
tional activity was seen predominantly in cells with morphologic features of
maturation. This second patient, who did not have extramedullary involvement
at the time of diagnosis, died early during induction therapy of bacterial pneu-
monia and septicemia. Serious infection occurred despite the presence of large
numbers of cells with documented ability to phagocytize bacteria, suggesting a
bactericidal defect.

Using a microbicidal assay which allowed simultaneous assessment of phago-
cytosis and bacterial killing by glass-adherent mononuclear cells, Cline has
described a disparity between the phagocytic and bactericidal capacities of
malignant monocytes. Monocytes from patients with myelomonocytic leu-
kemia ingested four species of bacteria normally but were distinctly inferior to
normal controls in their ability to prevent intracellular growth of these organ-

isms. The killing defect was particularly prominent with gram-negative organisms. Furthermore, the intracellular but viable bacteria were partially protected from the bactericidal action of antibiotics with which they were incubated. Although bactericidal capacity was not quantitatively assessed in our study, no bacteriolysis of ingested *E. coli* was detected in electron micrographs. The nature of the intracellular, possibly enzymatic, defect has not been identified, but it would appear that the neutropenic patient with acute monocytic leukemia remains with an increased risk of infection despite the presence of phagocytically competent cells.

Lichtman and Weed recently described a patient with monocytic leukemia who also had pronounced extramarrow infiltration. They were able to distinguish two populations of circulating monocytoid cells. The first category included cells which were weakly adherent to cover slips, weakly phagocytic, poorly deformable, highly negatively charged, and morphologically similar to blasts. The second group consisted of more morphologically mature monocytoid cells which adhered and phagocytized well, were as distensible as normal cells, and which had a reduced negative-charge density, similar to normal cells. These investigators felt that it was these properties associated with cytoplasmic maturation which enabled increased tissue entry to take place.

Although skin windows were not performed, a pleural effusion which developed in their patient contained only promonocytes at a time when blasts were predominant in the peripheral blood. This is analogous to our pretreatment skin window finding that only cells which appeared to have undergone some morphologic maturation were able to migrate. In contrast to Lichtman’s and Weed’s findings, less differentiated, primitive cells from our patient could both ingest particles and bacteria and adhere to nylon fibers. There is no apparent explanation for this difference. Still, the two studies are similar in that they both support the concept that, at least in some patients with acute monocytic leukemia, there exists a population of morphologically and functionally more differentiated cells capable of extravascular migration and division. This may in part account for the greater incidence of tissue involvement in patients with monocytic leukemia.

**REFERENCES**

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