Peripheral Cytoplasmic Characteristics of Leukocytes in Monocytic Leukemia: Relationship To Clinical Manifestations

By Marshall A. Lichtman and Robert I. Weed

The increased prevalence of tissue infiltration in some cases of monocytic leukemia may represent an enhanced ability of monocytes for tissue entry, continued cell proliferation, an increased life span in the tissues, or a combination of these three factors. Studies in this laboratory have shown that the normal myeloblast undergoes a process of cytoplasmic maturation that adapts it for marrow egress, tissue emigration, and particle ingestion. These include a reduction in surface negative charge density, increased ability to adhere to negatively charged surfaces, increased cytoplasmic deformability, more rapid motility, and enhanced phagocytic rate. In a subject with monocytic leukemia in whom tissue infiltration was a striking feature of the clinical disease, blood monocytes were broadly distributed in regard to their peripheral cytoplasmic characteristics. One portion of the cell population had a high surface negative charge density, was weakly adherent, weakly phagocytic, and poorly deformable, akin to blast cells. A second portion of the cell population had lower surface negative charge density, was adhesive, phagocytic, and readily deformable, akin to mature blood phagocytes. In five patients with myeloblastic leukemia in whom tissue infiltration was not prominent and in whom 92% of cells were myeloblasts, the blood leukocytes had a more homogeneous distribution of surface properties, and these properties were characteristic of immaturity. The increased prevalence of tissue infiltration in some cases of monocytic leukemia may be due to the presence of cytoplasmic maturation, which normally adapts the monocyte for tissue entry.

MORPHOLOGIC VARIANTS of acute granulocytic leukemia have been of long-standing interest to hematologists, although the biologic implications of these variations have not been clearly elucidated and the clinical distinctions, if any, have been minimal. The nosology of leukemia is undergoing reordering in light of a more accurate understanding of the pathogenesis of the disease. In these schemes, the morphologic variations in granulocytic leukemia appear to reflect variable expressions of a single underlying disturbance.1-3

Certain clinical variants of acute leukemia that have specific cytologic
LEUKOCYTES IN MONOCYTIC LEUKEMIA

Fig. 1. Examples of leukocytes in blood of patient F.L. stained with Wright's stain. Panels A and D represent myeloblasts with high nuclear-cytoplasmic ratios and faint nucleoli (lost in reproduction). Panels B and E represent larger cells with lowered nuclear-cytoplasmic ratios, more reniform nuclei and inapparent nucleoli (promonocytes). Panels C and F represent morphologically more distinct promonocytes. Larger cells with still lower nuclear-cytoplasmic ratios and reniform nuclei. (Original magnification Panels A-C, × 1000; Panels D-F, × 1250.)

characteristics have been associated with specific clinical characteristics, for example, the prominent extravascular accumulation of cells in “Schilling” type monocytic leukemia. The following report describes studies of the biophysical properties of leukocytes from a patient with monocytic leukemia in whom extravascular cellular infiltration was unusually prominent during the disease. Evidence for cytoplasmic differentiation of leukemic monocytes is presented. Cytoplasmic differentiation may have been an important factor in the prominence of extravascular accumulation of leukemic cells, since such maturation may adapt phagocytic leukocytes for tissue entry.

CASE REPORT

F. L., a 20-yr-old man, was admitted to Strong Memorial Hospital-University of Rochester Medical Center on October 9, 1968 after a 6-wk history of swollen gingiva, lightheadedness, palpitations, and night sweats. Physical examination identified marked gingival swelling, pallor, and moderate splenomegaly. The white cell count was 94,001/μl with 89% of cells either myelomonoblasts or promonocytes. The bone marrow aspirate was markedly hypercellular (22% M—E layer) with over 85% of cells having characteristics of myelomonoblasts or promonocytes (Fig. 1). Thrombocytopenia and anemia were marked. Histochemical studies of the patient's cells, including periodic acid Schiff, peroxidase, and sudan
black stains, were indicative of monocytes as described by Hayhoe. The patient was treated with packed red cells, platelet concentrates, and cyclophosphamide, vincristine, and cytosine arabinoside i.v., weekly. Gingival enlargement required external irradiation (900 R), which resulted in reduction of gingival infiltration. The patient developed pancytopenia after drug therapy, which was continued as an outpatient. Despite intermittent combination treatment with a Vinca alkaloid, alkylating agent, and an antimetabolite, the patient was readmitted on February 3, 1969 with a white cell count as high as 178,000/μl with 85–90% of cells, myelomonoblasts or promonocytes. During this admission, gingival, laryngopharyngeal, pulmonary, pleural, perianal, and cutaneous cellular infiltrates were present. The hypopharyngeal infiltrates had lead to vocal cord infiltration and destruction and compromise of the laryngeal lumen, eventually requiring tracheostomy. Cutaneous cellular infiltrates were prominent and repeatedly developed at venepuncture sites until the white cell count was reduced by chemotherapy. Examination of plural fluid with Wright's stains revealed monocyctic cells. The patient died on March 3, 1969. Prior to treatment and during relapse certain biophysical properties of the patient's cells were studied.

MATERIALS AND METHODS

Leukocyte isolation

Peripheral blood was obtained from normal subjects, subjects with neutrophilia, but without primary disorders of leukopoiesis, untreated patients with acute granulocytic leukemia in which blast cells comprised greater than 92% of cells, and patient F.L. Blood was anticoagulated with sodium heparin (15 U/ml) and allowed to sediment by gravity in plastic 1 ml tubes. Cell suspensions in plasma, so obtained, were diluted with autologous plasma as required for specific measurements. To obtain cell populations containing predominantly neutrophils, particularly for studies of cell electrophoresis, blood was obtained from subjects with neutrophilia in which PMN's composed more than 93% of circulating cells.

Cell adhesion

Adhesiveness of leukocytes was measured by two techniques. The column technique was a modification of previously described methods and provided the advantage of easier morphologic identification of cells that traversed the column. Measured volumes of dilute cell suspensions in plasma were added to a 3 ml plastic syringe containing a pledget of glass wool at its tip and 2 ml of glass beads (diameter 0.45–0.55 mm) so as to fill the void volume. After standing for 15 min at 37°C, elution was performed with a 3 ml sample of autologous plasma applied at the rate of 1 drop every 2 sec. Numerical cell counts and differential cell counts were made of the original cell suspension, and eluates and per cent adherence of specific cell types was calculated.

Adhesiveness of cell suspensions was also measured in a chamber composed of 0.016 inch brass plate 1¼ X 1½ inch with a central hole that was covered on both sides by glass or plastic cover slips as desired and attached to the brass slide with melted wax as previously described. This method allowed the application of detaching forces to the adhesive union of cells to substratum. Cell suspensions could be introduced into the chamber through an extension of the central hole left uncovered by the cover slip, after which the small residual entry and air exhaust orifices were sealed with wax. Cells were allowed to settle on the lower cover slip for 15 min at 37°C. The chambers were inverted, placed in the bottom of a large centrifuge cup, covered with water, and a peak instantaneous detaching centrifugal force of 75 g applied. The number of residual cells attached to the upper cover slip (adherent) and resting on the lower cover slip (nonadherent) were enumerated under phase-contrast microscopy at up to 800× magnification in at least ten randomly selected fields (75–150 cells), and per cent adherence was calculated. Thereafter, cover slips could be removed, air dried, stained with Wright's stain, and examined. Special care had to be used to recover nonadherent cells during detachment of the lower cover slip.
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Cell electrophoretic mobility

Cell electrophoretic mobility was measured at 23°C in a rectangular cuvette as described by Fuhrmann and Ruhenstroth-Bauer. Cell electrophoretic mobility was measured at 23°C in a rectangular cuvette as described by Fuhrmann and Ruhenstroth-Bauer.7 Leukocytes were washed once in Sorensen's phosphate-buffered sorbitol pH 7.2, (1 part 1/15 M phosphate buffer plus 4 parts 5% sorbitol), and electrophoretic mobility measured in the same suspending medium.8 The time in seconds required for a leukocyte to traverse 147 μ in a field strength of approximately 19.0 V/cm at a current of 3 mA was recorded. Mobility rate was expressed as μ/sec/V/cm. Each cell was measured twice, with reversal of polarity after the first measurement. In each experiment, measurements were made at the anterior and posterior stationary level of the electrophoresis cuvette.

Cell deformability

Leukocytes from blood and marrow were diluted in autologous cell-free plasma, so as to provide a cell density of two to six leukocytes per microscope field. Deformability of leukocytes was measured with a cell elastimeter with micropipette orifice having a measured internal diameter as previously described.9 Marrow leukocytes were studied with a Zeiss phase-contrast microscope with a long working distance objective and calibrated eyepiece micrometer. Individual cells could be examined at magnifications of up to 1000X. The temperature of samples was maintained at 36–38°C with a Sage Instrument air curtain and thermistor probe. The deformability of individual cells was judged by the total negative pressure required to aspirate a hemispherical bulge of cell into the pipette.

Cell motility rate

Leukocyte suspensions in plasma were placed in microcapillary tubes (Drummond Scientific) and centrifuged in a Clay-Adams microhematocrit centrifuge for 5 min. The microhematocrit tubes containing sedimented leukocytes were placed vertically in a specially made plastic holder, and migration distance of the advanced edge of the buffy coat was measured in triplicate eyepiece micrometer.5 The temperature of the microcapillary tubes was maintained at 36–38°C with a Sage Instrument air curtain.

Phagocytosis

Ten microliters of a known number of sterile monodispersed latex spherules of 1.3 μ diameter, prepared in Hank’s balanced saline solution (HBSS), were added to a 2 ml leukocyte suspension prepared aseptically from heparinized blood or marrow samples and diluted with autologous plasma, such that a leukocyte/particle ratio of 2 to 1 was obtained. Cell suspensions containing latex particles were incubated at 37°C with gentle tumbling. Samples were removed at 1 hr, and carefully prepared cover slip smears were made at these times and stained with Wright’s stain. For some experiments, increased proportions of latex particles were used. The number of cells with ingested latex particles divided by the total number of cells of that particular morphologic type represented the proportion of cells demonstrating particle ingestion.

RESULTS

Adhesiveness

PMN’s and monocytes from normal donors suspended in autologous plasma at 37°C exhibited a mean of 96% and 97% adherence to plastic cover slips, despite the application of detach ing forces of up to 75 g (Table 1). Bone marrow cells from donors without abnormalities of leukopoiesis had about 58% of cells adherent. Adherent bone marrow cells were monocytes, PMN’s, bands, and metamyelocytes, predominantly. Less mature granulocytes have a decreased propensity to adhere, and the nonadherent cells were myeloblasts,
Fig. 2. The distribution of the rate of anodal electrophoretic mobility of single cells. Mature granulocytes and monocytes have a reduced surface negative charge density and a reduced rate of mobility. Leukemic monocytes from F.L. had a population of cells with a reduced negative charge density more analogous to mature, normal phagocytes than to leukemic blast cells.

Fig. 3. The distribution of the negative pressure to aspirate the periphery of cell cytoplasm into a hemispherical deformity. Mature granulocytes and monocytes require low negative pressures to aspirate a tongue of peripheral cytoplasm. Leukemic and normal blast cells require very high pressures to deform. Leukemic monocytes from F.L. had a population of cells that required low deforming pressures to aspirate cytoplasm.
promyelocytes, large myelocytes, and some small myelocytes. Less than 6% of myeloblasts from patients with acute leukemia were adherent. PMN's in such patients were adherent, but the proportion that adhered was less than normal. However, patients with acute leukemia were selected with a very low proportion of circulating PMN's (less than 8%), and hence the number of PMN's enumerated was too small to be certain of this difference. In patient F.L. with monocytic leukemia, adhesiveness studied by the column method indicated that 61% of cells were adherent. Thirty-eight and 58% of cells were adherent by the chamber method on two occasions, despite detaching forces of 75 g. Cells that were obviously monocytic morphologically were nearly all adherent. Nonadherent cells were composed predominantly of blast cells (myelomonoblasts).

**Cell electrophoretic mobility**

Normal marrow cells had mobilities ranging from 0.90 μ/sec/V/cm to 2.22 μ/sec/V/cm (Fig. 2). Glass bead filtration of marrow, so as to exclude mature granulocytes, resulted in mobilities ranging from 1.40 μ/sec/V/cm to 2.21 μ/sec/V/cm. Whereas mobility of PMN's, monocytes, and bands from blood ranged from 0.90 to 1.60 μ/sec/V/cm, myeloblasts from subjects with acute granulocytic leukemia had a mobility ranging from 1.35 to 2.50 μ/sec/V/cm, a distribution similar to normal immature granulocytes. The cells from the subjects with monocytic leukemia had a distribution of mobilities from 1.00 to 2.10 μ/sec/V/cm, overlapping the range for blasts and mature monocytes. Individual cellular identity was not possible, but it is presumed that the more slowly migrating cells (with reduced surface negative charge density) were promonocytes that exhibited morphologic characteristics of maturity.

**Cellular deformability**

The distribution of pressures required to aspirate a hemispherical deformity from normal marrow cells into a micropipette of 3.5 μ internal diameter is shown in Fig. 3. Blasts and progranulocytes required high pressures for slight deformation, myelocytes required less negative pressure, and PMN's and monocytes required the lowest negative pressures in order to aspirate a segment of cell cytoplasm into the pipette. The pressure required to aspirate leukemic myeloblasts into the micropipette was high, although similar to the pressures required to deform normal blast cells. In the case of monocytic leukemia studied, cell deformability was more nearly like normal marrow, in that a sizable population of cells were deformable at low negative pressures. These cells could be identified morphologically as monocytic, whereas the cells requiring larger negative pressures to deform were predominantly myelomonoblastic.

**Phagocytosis**

Under the conditions of study in these experiments, 36% of PMN's and 29% of monocytes showed particle ingestion, whereas normal myeloblasts and acute leukemic myeloblasts had a median of 0 and 2.4% of cells with
Table 1. Adhesiveness and Phagocytosis of Leukocytes

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<th>Normal</th>
<th>Leukemic</th>
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<tr>
<td></td>
<td>PMN</td>
<td>Monocytes</td>
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<td>Adherent cells</td>
<td>96±</td>
<td>97</td>
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<td>(92–100)</td>
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<td>Phagocytosis cells</td>
<td>36</td>
<td>29</td>
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*From patients with acute granulocytic leukemia.
†Results of study prior to treatment and during relapse in patient F.L. Represent all abnormal cells in blood (myelomonoblasts and promonocytes).
‡Mean and range (in parenthesis).

Ingested particles, respectively (Table 1). In the cells from F.L., 25 and 21% of monocytes contained ingested particles after 60 min of incubation on two occasions. Ingestion was most prevalent in promonocytes and least prevalent in myelomonoblasts. In confirmation of this, monocytic cells in autologous plasma from F.L. were placed in the adhesiveness chamber at 37°C, and 1.3 μ diameter latex spherules were added so as to have a particle to cell ratio of 2 to 1. After 45 min, the chamber was inverted, and phagocytosis by adherent cells (on the top cover slip) and by nonadherent cells (on the bottom cover slip) was estimated by phase-contrast microscopic examination. Forty-nine per cent of adherent cells and 5% of nonadherent cells had ingested particles.

Migration rate

The migration of the advanced edge of the buffy coat in microcapillary tubes was nearly linear over the initial 60 min of observation. The rate of movement of cells from F.L. was more rapid (0.085 mm/hr) than that of myeloblasts from five patients with acute leukemia (0.010–0.035 mm/hr). Migration rates for normal monocytes were not studied, but PMN’s from six healthy subjects moved at a more rapid rate (0.13–0.31 mm/hr) than leukemic myeloblasts or leukemic monocytes.

DISCUSSION

Increasing evidence indicates that acute leukemia in man is a disorder that affects a primitive (stem) cell that is capable under normal circumstances of multivariate differentiation into erythroid, megakaryocytic, granulocytic, and monocytic cells. Current data suggest that the blood monocyte and tissue histiocyte (macrophage) are similar, if not identical, cells and have their origin in bone marrow. Moreover, the precursor of the monocyte is a primitive cell capable of undergoing multivariate differentiation. Differentiation of this primitive cell into one of several alternative cell types may be directed by inducers whose regulation and mechanism of action awaits further elucidation. It could be predicted, however, that their mode of action will be similar to other hormonal agents that activate latent nuclear genomes.
Evidence has been marshaled to indicate that the leukemic stem cell also may undergo several degrees of maturation. In the least successful degree, the leukemic blast cell decreases in volume, has a reduced propensity for cell division, and may be unable to undergo further proliferation, its life ending in eventual cytolysis. In such circumstances, its ability to mimic the functional capacity of a fully differentiated phagocyte (i.e., perform chemotaxis and phagocytosis) is usually nil. The most successful form of leukemic maturation is that seen in chronic granulocytic leukemia, where the number of mature cells that accumulate exceeds normal and the functional capacity of mature cells is nearly normal. Between these extremes every degree of partial, morphologic, and functional differentiation may occur highlighting the quantitative nature of “leukemicness”.

In this report, we provide further evidence for maturation of leukemic blast cells into cells with functional characteristics resembling normal mature monocytic phagocytes. The presence of $9 \times 10^7 - 1.7 \times 10^8$ monocytes/ml of blood, the persistence of nucleoli in monocytes that appeared partially differentiated morphologically (i.e., low nuclear/cytoplasmic ratio, reniform or segmented nucleus, gray-blue abundant cytoplasm, and cell spreading patterns characteristic of mature monocytes by phase-contrast microscopy), the histochemical characteristics of the myelomonoblasts and promonocytes, and the clinical course provide evidence that the cells under study were leukemic monocytes. A significant proportion of cells in the blood of patient F.L. had peripheral cytoplasmic characteristics indicative of maturation from a myelomonoblast. These included a lower surface negative charge density as reflected in a slower anodal electrophoretic mobility, an increased ability to adhere to a substratum, and heightened cytoplasmic deformability. As is the case of the normal maturation of phagocytes derived from the myeloblast (PMN’s and monocytes), the patient’s leukemic monocytes had a motility rate and phagocytic capacity that was superior to nonmonocytic leukemic blast cells. The role of the biophysical changes in peripheral cytoplasm during maturation of the normal myelomonoblast in the development of functional capacity of the cell have been discussed by us in previous reports.

That normal egress of phagocytes from circulation to tissue is dependent on prior maturation has been suggested by cytokinetic studies. Our studies of the biophysical properties of the cytoplasm of immature marrow cells have indicated that the blast cell is poorly equipped for entry into extravascular tissues by normal pathways, since it can neither adhere to substrata (endothelium) nor deform and migrate efficiently through the restrictive spaces between endothelial cells. The size and markedly heightened intrinsic viscosity and rigidity of leukocytes, particularly leukemic blast cells as compared to normal red cells, may explain their predisposition to entrapment in the microcirculation and sinusoidal organs. Entrapment of leukemic blast cells in the microcirculation with stasis and vascular damage can lead to invasion of vessel walls and a pathologic mode of tissue entry. Moreover, even if only very small percentages of blast cells manage to leave the circulation by “normal” routes, when present in large numbers in the circulation, the absolute number...
leaving per unit time may still be consequential in setting up tissue foci, since they may have the capacity to reenter the cell mitotic cycle, to divide, and thereby to colonize extravascular spaces. This presumably explains the tissue foci of leukemic cells observed in myeloblastic leukemia, although the role of partial cytoplasmic maturation and the ability to gain access to the tissue by normal routes must also be considered. The ability to reenter a proliferative phase after leaving the circulation could then result in varying degrees of tissue accumulation, depending on rate of tissue entry, generation time of blast cells in the tissue, rate of cell death, and perhaps rate of tissue exit. The ability of monocytes to undergo partial maturation so as to develop characteristics that facilitate tissue entry (a reduced surface negative charge, increased adhesive ness, deformability, and motility) should increase their exit from the circulation. In the patient F.L., tissue infiltrations were indeed striking. In addition to gingival and perirectal infiltrates (the former requiring radiotherapy for control) cutaneous, pulmonary, and laryngeal infiltrates were more prominent clinically than usually seen in patients with leukemia. Of particular interest was the distribution of cells in the pleural fluid, which when stained with Wright’s stain contained leukocytes that were composed primarily of pro-monocytes, whereas the blood at that time contained a high percentage of myelomonoblasts. This observation is similar to the results of studies in granulocytic leukemia in which more mature granulocytes enter exudates.\textsuperscript{11,12}

The leukemic blast cells in blood are composed predominantly of cells in G\textsubscript{n}, that is not in the process of mitosis, although most may be capable of reentering the cell mitotic cycle under the proper circumstances.\textsuperscript{13} The ability to continue cell division after circulatory egress also contributes to tissue accumulations, but the marked leukemic cellular infiltrates that developed rapidly at venepuncture sites in F.L. could not be accounted for by cell division alone, based on the most rapid generation times reported in acute leukemia.\textsuperscript{13} The longevity of monocytes in the tissues\textsuperscript{10} may also play an important role in tissue accumulations. In contrast to neutrophils that have been estimated to remain intact only a few hours after tissue entry, monocytes probably survive for an extended period of time. Leukemic monocytes may have an even lengthened tissue life span independent of their ability to divide.

The net effect of these several factors determines the tissue accumulation of cells. Our studies indicate that cytoplasmic maturation is an important feature of some cases of monocytic leukemia and thereby is a factor in the extent of extravascular accumulation of cells, since such maturation favors the rate of tissue entry.

The enhanced phagocytosis of cells in monocytic leukemia as compared to leukemic myeloblasts has been known for decades\textsuperscript{18} and may be another index of their cytoplasmic maturation.\textsuperscript{5} Human leukemic myeloblasts from patients with the accelerated phase of chronic granulocytic leukemia have been shown to be capable of bacterial particle ingestion in vitro. This also has been interpreted as evidence of partial cytoplasmic maturation.\textsuperscript{19}

The ability of large, poorly deformable leukemic blast cells to enter the circulation in abundance in leukemia may be due in part to a breakdown of the restrictive barrier thought to play a role in regulating release of marrow cells.\textsuperscript{5,9,20}
ACKNOWLEDGMENT

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