Rheology of Leukocytes, Leukocyte Suspensions, and Blood in Leukemia

POSSIBLE RELATIONSHIP TO CLINICAL MANIFESTATIONS

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A B S T R A C T Suspensions of leukemic lymphocytes and myeloblasts and blood of leukemic patients were studied to examine (a) the effect of leukemic cells on blood viscosity and (b) the ability of leukemic cells to traverse channels of capillary diameter. The viscosity of suspensions of leukemic cells was dependent logarithmically on (a) shear strain rate and (b) cytocrit, although, suspensions of small lymphocytes and of myeloblasts had a similar viscosity at equivalent shear rates and cytocrit. The minimum apparent viscosity (MAV) of leukemic cells and red blood cells, measured over shear rates of 2.3–230 s^{-1} was dependent logarithmically on cytocrit. However, MAV was slightly greater for leukemic cells than for red cells at cytocrits up to 20%. At cytocrits above 20%, MAV of leukemic cells increased more rapidly than that of erythrocytes. For example, at a 15% cytocrit MAVwbc (1.85 centipoise) was only slightly greater than MAVrbc (1.59); whereas, at 45% cytocrit MAVwbc (14.9) was markedly greater than MAVrbc (3.81).

The blood of subjects with leukemia with marked elevation of leukocyte concentration (leukocrits of 6–32%) had 24% higher mean MAV (3.72) than blood with a similar total cytocrit composed of red cells (3.00). A negative correlation was present between leukocrit and erythrocrit in chronic lymphocytic ($r = -0.82$) and chronic granulocytic ($r = -0.81$) leukemia. Therefore, the modest increase in whole blood MAV in leukemia can be explained by (a) the negative association of leukocrit and erythrocrit and (b) the rarity of leukocrits over 20% and total cytocrits over 45%. However, the MAV of blood of leukemic patients was 71% greater than expected on the basis of their packed red cell volume. Hence, the ratio of hemoglobin concentration (O$_2$ carrying capacity) to MAV was abnormally low in the subjects with leukemia studied.

Individual leukemic leukocytes were nearly rigid. The mean deformability index (DI) of leukemic myeloblasts (1.22; 1.18) and lymphocytes (1.22; 1.40) as measured by filtration and elastometry, respectively, at 50 mm H$_2$O negative pressure, approached that of a rigid body (1.0) as compared to red cells studied by filtration (3.09) or elastometry (4.23). The ability of leukemic cells to traverse nucleopore filter or micropipette channels was related to cell diameter. The relevance of the rheology of leukemic cells to the interruption of blood flow and of tissue oxygen delivery and thereby to clinical manifestations of leukemia is considered.

INTRODUCTION

The importance of erythrocytes and plasma proteins, especially fibrinogen, as determinants of normal blood viscosity has been studied extensively (1–6). Although the influence of normal numbers of leukocytes on blood viscosity measured in vitro is trivial (7, 8), in leukemia large accumulations of leukocytes may influence the viscosity and thereby the flow of blood (8–11). Earlier studies of leukemic blood were subject to important artifacts resulting from the use of capillary tube viscometers. A more recent study using modern instrumentation concluded that the blood in leukemia is not hyperviscous (12). However, the viscosity of blood in leukemia may be abnormal in relation to its oxygen carrying capacity (hemoglobin concentration), assuming that a reduction in the former compensates, in part,

for a reduction in the latter (13). If flow is maintained with an increase in viscosity, appropriate changes in peripheral resistance or cardiac output must ensue (14). In addition, a greater intrinsic viscosity of leukemic cells may be of consequence in the microcirculation since the leukemic blast cell is not capable of normal circulatory egress (15), possibly because it is neither able to adhere to a substratum and by inference to vascular endothelium nor to deform and migrate through small diameter pores (16) and by inference intercellular spaces of vascular endothelium. Also, the leukemic cell has a diameter that exceeds the diameter of some microcirculatory channels requiring the cell to be deformable for passage. These characteristics could lead to deleterious changes in regional blood flow.

The following studies were made (a) to further characterize the viscosity of suspensions of leukemic leukocytes, (b) to examine the relative contribution of leukocytes to blood viscosity in subjects with leukemia, (c) to examine the cytocrit, a primary determinant of blood viscosity, and the relationship of packed red cell volume (erythrocrit) to packed leukocyte volume (leukocrit) in subjects with leukemia, and (d) to examine the capability of individual leukemic leukocytes to traverse channels with small diameters (1–10 μm), by studying their ability to negotiate nucleopore filters and glass microcapillary tubes. The possible effect of leukemic leukocytes on blood flow is considered.

METHODS

Preparation of cell suspensions. Venous blood from normal subjects and patients with acute (AGL)[1] and chronic (CGL) granulocytic leukemia and acute (ALL) and chronic lymphocytic leukemia (CLL) was collected in sodium heparin (14 U/ml). In order to prepare leukocytic suspensions, blood from subjects with leukemia with leukocrits of 6–32% was sedimented at 25°C at 1 g for 20–45 min depending on sedimentation rate, and the supernatant removed and resedimented at 1 g for 15 min. The supernatant cell suspension was removed, centrifuged at 75 g and resuspended to varying leukocrits in autologous plasma previously centrifuged at 10,000 g for 20 min to remove platelets. Red cell suspensions, from which leukocytes and platelets had been removed by aspiration of the buffy coat and plasma after centrifugation at 250 g, were resuspended in autologous platelet-free plasma to desired erythrocrits.

Blood cytocrit, cell counts, and hemoglobin concentration. Packed cell volume (cytocrit) was measured in quadruplicate in microhematocrit tubes (Arthur H. Thomas Co., Philadelphia, Pa.) after 8 min centrifugation at approximately 10,000 g in an International Microcapillary centrifuge, model MB (International Equipment Company, Needham Heights, Mass.). Erythrocyte and leukocyte counts

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[1] *Abbreviations used in this paper:* AGL, acute granulocytic leukemia; ALL, acute lymphocytic leukemia; CGL, chronic granulocytic leukemia; CLL, chronic lymphocytic leukemia; DI, deformability index; MAV, minimum apparent viscosity; MCV, mean corpuscular volume; RBC, red blood cells, WBC, white blood cells.

were made with Celloscope cell counter (Particle Data, Inc., Elmhurst, Ill.). Hemoglobin was measured as cyanmethemoglobin at a wavelength of 540 nm using a Bausch and Lomb Spectronic-20 photometer (Bausch & Lomb Incorporated, Rochester, N. Y.).

Viscosity. Viscosity of (a) red cell suspensions, prepared to desired erythrocrits in autologous plasma, (b) whole blood of subjects with AGL, CGL, ALL, and CLL with high leukocrits, (c) leukemic leukocyte suspensions prepared to desired leukocrits in autologous plasma, and (d) platelet-free plasma was measured at 37°C in a Wells-Brookfield LVT cone-plate micro-viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, Mass.) at shear rates from 2.3 to 230 s^{-1}. The instrument was calibrated by use of Brookfield viscosity standards at 37°C. Apparent viscosity (η) was calculated in centipoise from the formula:

$$\eta = \frac{\text{Shear stress (dynes/cm}^2)}{\text{Shear strain rate (s}^{-1})} \times 100.$$

Minimum apparent viscosity (MAV) was determined by squaring the slope of the regression of the square root of shear stress on the square root of shear rate (double square root plot of Casson [17]) at a specific cytocrit over a range of shear rates from 2.3 to 230 s^{-1}.

Filterability. Leukocytes and red cells were suspended in 285 mosM phosphate buffered sodium chloride, pH 7.4, at a cell concentration of 6–8 $\times$ 10^4 cells/ml. 4 ml of this suspension containing a known number of cells was filtered through high density (1–4 $\times$ 10^5 pores/cm^2) Nucleopore membrane filters of 2.5 cm diameter (General Electric Company, Pleasanton, Calif.) of known mean pore diameters (1, 2, 3, 5, or 8 μm) at room temperature. These filters are approximately 12 μm in thickness. The filter was supported in a microsyringe filter holder with extension barrel containing plastic gaskets (Millipore Filter Corp., Bedford, Mass.). Filtration was performed without negative pressure (mean pressure head 10.5 mm H$_2$O) and with a total of 50 mm H$_2$O negative pressure applied by Macro model M air pump (J. B. Maris Co., Bloomfield, N. J.) and quantified by a water manometer. The percent of cells filtered was determined by the formula:

$$\frac{\text{no. of cells in filtrate}}{\text{no. of cells applied to filter}} \times 100.$$

Cell volumes. Red cell diameters were measured directly by phase contract microscopy. Cell volume distributions were measured with a 512 channel particle analyzer (Nuclear Data, Inc., Palatine, Ill.), attached to a Celloscope cell counter (Particle Data, Inc.). Absolute mean cell volume of leukocytes was determined by direct measurement of the diameter of 50 cells, selected, randomly, using a Zeiss phase contrast microscope (Brinkmann Instruments, Inc., Westbury, N. Y.) and a calibrated eyepiece micrometer at a magnification of $\times$ 800. Mean volume was determined from the formula for the volume (V) of a sphere, $V = \pi d^3/6$ where d represents cell diameter. The volume distribution of cells was converted to the distribution of cell diameter by solving the volume formula for d at nine points on the volume distribution curve. Red cell volumes were measured on the Nuclear Data, Inc. particle volume analyzer using CBC-trol particles of known volume (Charles Pfizer & Co., Inc., New York) as a reference standard.

Elastometry. Leukocytes obtained by gravity sedimentation of red cells and removal of supernatant plasma, were diluted with autologous plasma to provide a density of 8–10
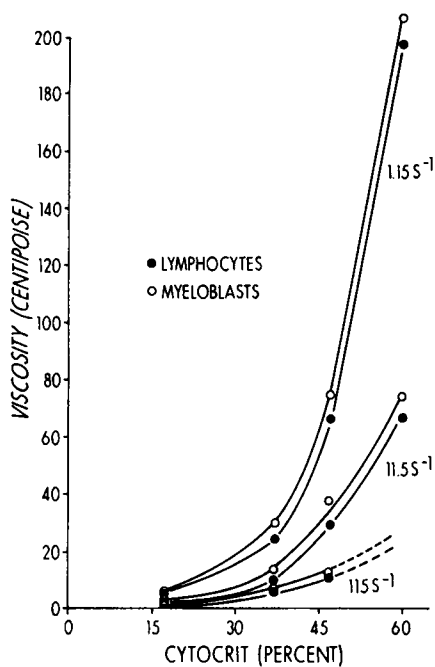
FIGURE 1 The viscosity of leukemic myeloblasts and lymphocytes is compared at three rates of shear. The dependence of viscosity of leukocyte suspensions on cytocrit and shear rate is evident. Little difference in viscosity of the two cell types was seen at similar cytocrit and shear rate.

cells per microscope field. Deformability of cells was measured with a cell elastometer having a micropipette orifice of measured internal diameter using Zeiss phase-contrast microscope with a long working distance objective and
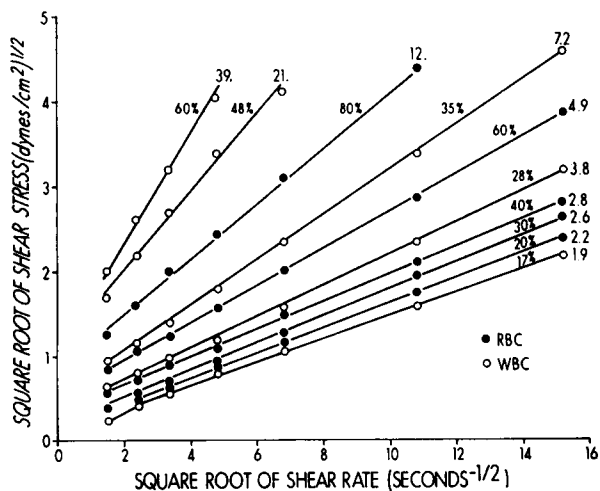

FIGURE 2 Casson plots of pure leukocyte and red cell suspensions in autologous plasma are shown. The percent cytocrit represented is indicated. The numbers at the end of each curve represent the minimum apparent viscosity in centipoise (see Methods). The slopes were obtained by regression analysis. The curves were all shown statistically to satisfy the linearity hypothesis.

calibrated eyepiece micrometer as previously described (18). Individual cells were examined at magnification up to × 1000 and at room temperature. The deformability of cells in this study is represented by the proportion of leukemic lymphocytes or myeloblasts which could enter in their entirety a micropipette orifice of a given internal diameter when 50 mm of H_2O negative pressure was applied to the micropipette.

RESULTS

The viscosity of myeloblasts (mean corpuscular volume [MCV] = 395 μm^3) and CLL lymphocytes MCV = 189 μm^3) was similar at equivalent cytocrits (Fig. 1). The marked shear dependence of the viscosity of suspensions of both leukocyte types is evident, as is the exponential increase in viscosity with increasing cytocrit. The dependence of leukocyte viscosity on packed cell volume and on shear rate was present when leukocytes were suspended in Hanks's salt solution as well as plasma. The regression of the logarithm of viscosity of leukocyte suspension on cytocrit at a given shear rate is approximately linear (data not shown).

The square root of shear stress of leukocyte and erythrocyte suspensions had a linear dependence on the square root of shear rate between rates of 2.30 and 230 s^{-1} (Fig. 2). At leukocrits of 48 and 60%, and erythrocrits of 80%, the relationship may be slightly curvilinear, however, the observed points did not deviate significantly from linearity. The double square-root plot provides a useful single numerical value for apparent viscosity over a wide range of shear rates, recognizing that the linearity assumption may not be strictly valid. The yield stress of cell suspensions cannot be extrapolated from these lines since the relationship of shear stress to shear rate may be different at shear rates below 2.3 s^{-1} (19). Merrill and Pelletier (20) and Merrill (21) have discussed the limitations involved in the use of the Casson equation over different ranges of shear rate. In our studies the double reciprocal plot so closely approximated linearity between 2.3 and 230 s^{-1} that it was used to apply a single index of apparent viscosity.

MAV calculated from the slope of Casson plots also increased logarithmically with increasing cytocrit when suspensions of erythrocytes and leukocytes were examined (Fig. 3); however, a complex relationship existed for leukocytes. The dependence of the viscosity of leukocyte suspensions on cytocrit was slightly greater than that of erythrocyte suspensions up to a cytocrit of 20%, after which leukocyte MAV increased at a more rapid rate than that of erythrocytes.

The MAV of whole blood from subjects with leukemia (3.72±0.36, mean ±SE) was significantly ($P < 0.01$) although only modestly (24%) greater than blood with an identical total cytocrit composed of erythrocytes (3.00±0.057) (Table I). Moreover, with the notable exception of one subject with CGL who had a leuko-

crit of 32% (Table I) and a blood MAV of 7.8 centipoise, the whole blood viscosity of patients with leukemia did not exceed that of normal blood with a 47% erythrocrit (see Fig. 3). However, if one considers the blood viscosity of the patients studied in terms of its relationship to oxygen carrying capacity (hemoglobin concentration) a quantitatively greater effect is observed. The MAV of leukemic blood (3.72±0.36) was 76% greater than expected for blood (2.11 ± 0.033, $P < 0.001$) with an erythrocrit identical to the erythrocrit of the subjects with leukemia studied (Table I). When the relationship of hemoglobin concentration to MAV at varying erythrocrits was examined, the curve described an optimum at about an erythrocrit of 32% (Fig. 4). At low erythrocrits, the reduction in viscosity does not compensate proportionately for the reduction in oxygen carrying capacity while at high erythrocrits the increase in oxygen capacity does not compensate proportionately for the increased viscosity. The ratio of hemoglobin concentration to MAV was markedly reduced for 13 leukemia patients with elevated leukocrits who were studied (Fig. 4). This was the result of an increase in MAV produced by leukocytes without a concomitant increase in oxygen carrying capacity.

The erythrocrit of 78 patients with chronic leukemia was correlated with leukocrit at the time of diagnosis. A highly significant negative correlation ($P < 0.001$) before specific therapy was present (Fig. 5). In subjects with CGL, a reduction in erythrocrit of 1.08% occurred for every 1.0% increment of leukocrit. In subjects with CLL, a 1.48% reduction in erythrocrit occurred with every 1.0% increment in leukocrit.
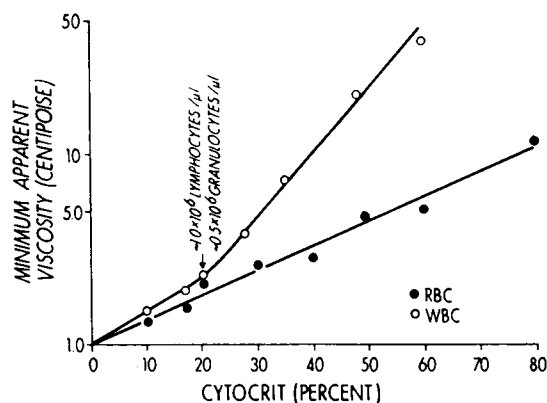

FIGURE 3 The minimum apparent viscosity of leukocytes and erythrocytes suspended in plasma at varying cytocrits. An approximately linear increase in MAV occurs with increasing erythrocrit. The relationship is more complex for leukocytes. The rate of increase in MAV is linear; however, when the leukocrit approaches 20%, a more rapid increase in MAV occurs with increasing leukocrit. A 20% leukocrit represents about a half million leukemic myeloblasts and a million CLL lymphocytes per μl, based on an average MCV of 400 μm³ for leukemic myeloblasts and 200 μm³ for CLL lymphocytes.

Plasma viscosity was not significantly different when leukemia and normal subjects were compared. The MAV of plasma was 1.19±0.07 (mean ±SE). The curve relating MAV of cell suspensions to cytocrit in Fig. 3, intercepts the ordinate at a MAV of 1.0 when the cytocrit is zero. This is explained by the use of plasma as the suspending medium leaving a residual MAV of approximately 1.0 in the absence of cells. The absence

TABLE I
Minimum Apparent Viscosity of Leukemic Blood

Type of leukemia	Leukocyte count	Leukocrit	Erythrocrit	Observed MAV	Expected MAV	
					For cytocrit	For erythrocrit
	no./μm X 10³	%	%			
AGL	230	10.5	29.5	3.9	3.3	2.4
AGL	190	11.0	23.0	3.3	2.8	2.0
AGL	206	9.0	17.5	2.7	2.2	1.7
AGL	160	7.0	29.0	3.1	2.9	2.4
ALL	775	24.5	10.5	3.7	2.8	1.5
CGL	619	32.0	17.5	7.8	4.2	1.7
CGL	280	10.5	30.5	3.6	3.3	2.5
CGL	206	9.0	27.5	3.9	2.9	2.3
CGL	240	8.5	29.0	3.4	3.1	2.4
CGL	220	6.0	30.5	3.8	3.0	2.5
CLL	890	18.0	17.0	3.2	2.9	1.8
CLL	490	11.0	23.0	2.8	2.7	2.0
CLL	450	10.0	26.0	3.2	2.9	2.2
Mean ±SE				3.72±0.36	3.00±0.057	2.11±0.033

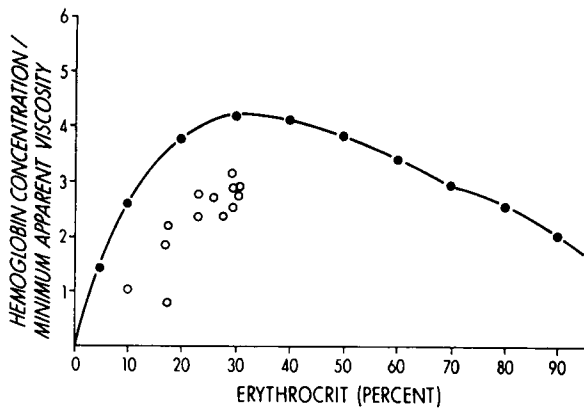MAV, minimum apparent viscosity (centipoise).

FIGURE 4 The relationship of the blood hemoglobin: blood viscosity ratio to packed red cell volume is depicted. This ratio has an optimum at an erythrocrit of about 32%. The 13 patients with leukemia studied have a markedly reduced hemoglobin/viscosity ratio (2.33±0.20; mean SE) at their own erythrocrit due to an increased leukocrit and thereby viscosity of their blood. Their oxygen carrying capacity (hemoglobin) to viscosity ratio was similar to blood with an erythrocrit of 3–14% (severely anemic) and to blood with erythrocrit of 65–95% (severely polycythemic) and significantly lower (40%) than expected for blood of a similar erythrocrit (3.78±0.14). It must be noted that cardiovascular adaptations and reduced hemoglobin oxygen affinity in leukemic subjects could shift their curve toward the expected for anemic subjects without extreme leukocytosis.

of a difference in plasma viscosity when normal and leukemia subjects were compared is in contrast with the
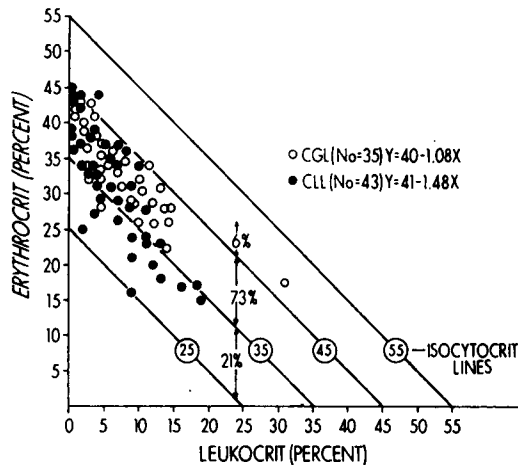

FIGURE 5 The correlation of erythrocrit with leukocrit in 78 cases of chronic leukemia. A highly significant negative relationship exists. Part of the variability is explicable by the effect of sex on erythrocrit, although this was not important enough to warrant displaying the relationship for each sex group. The sum of leukocrit and erythrocrit never exceeded 50% in these 78 cases and in only 6% of cases did total cytocrit exceed 45%. In 73% of cases the cytocrit was between 35 and 45%.

observations of Dintenfass (22) and in agreement with the observations of Steinberg and Charm (12).

Filterability. The filterability of red cells and leukemic cells through Nucleopore filters differed markedly (Fig. 6). A mean of 67% of normal red cells traversed a filter with 3.0 μm diameter pores at 50 mm H_2O negative pressure; and over 95% of red cells traversed a filter with 5.0 μm pores without additional negative pressure being applied. Filterability of leukemic cells was related to cell diameter. As cell diameter increased, the ability to traverse the filters decreased (Fig. 6). Leukemic lymphoblasts were larger and less readily filtered than leukemic small lymphocytes. Leukemic myeloblasts were of largest diameter and least filterable. Moreover, smaller myeloblasts filtered more readily than larger myeloblasts. In order to compare the filterability of cell populations of different size, the relationship of the median diameter of the cell population ($\bar{d}$), to the diameter of the pore traversed by 50% of cells (d_{50}) was used as an index of deformability. If a cell is a rigid body such that no deformation occurs, it will only traverse a pore with a diameter at least equal to that of the cell, resulting in a deformability index of 1.0, and indicating total rigidity at the pressure gradient applied. Red cells with a mean diameter of 8.15 μm have a deformability index as measured by filtration, 2.5 times that of leukemic myeloblasts of similar mean diameter (Table II). Leukemic myeloblasts and lymphocytes had a similar deformability index which approached total rigidity (1.22) at a negative pressure of 50 mm H_2O. The erythrocyte will traverse a filter pore 5.5 μm in diameter smaller than that of the average cell as compared to lymphocytes and myeloblasts which will traverse
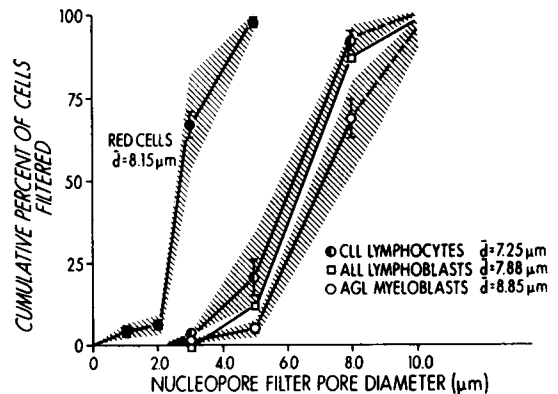

FIGURE 6 The percent of cells capable of being filtered through Nucleopore polycarbonate filters with pore diameters of 1, 2, 3, 5, and 8 μm. The shaded area represents the range of percent of cells filtered with 50 mm H_2O negative pressure being applied to the efferent side of the filter. The mean diameters ($\bar{d}$) of the cells studied are shown. Filtration of leukocytes was directly related to cell diameter.

TABLE II
Filterability and Elastometry of Leukemic Cells

	Mean Cell diameter ($\bar{d}$)	Pore diameter at which 50% of cells traverse* (d_{50})	Deformability index (DI)
	μm	μm	d/d_{50}
Nucleopore filterability			
Red cells (n = 6)	8.15±0.057	2.68±0.35	3.09±0.051
CLL lymphocytes (n = 6)	7.25±0.14	6.06±0.32	1.22±0.089
ALL lymphoblasts (n = 1)	7.88	6.4	1.21
AGL myeloblasts (n = 6)	8.85±0.18	7.22±0.21	1.22±0.021
Micropipette deformability			
Red cells	8.1	1.8	4.50
Red cells	8.3	2.1	3.96
CLL lymphocytes	6.4	4.4	1.45
CLL lymphocytes	7.2	5.3	1.35
AGL myeloblasts	8.3	7.1	1.17
AGL myeloblasts	8.9	7.8	1.18

* With 50 mm H₂O negative pressure applied to the filter and micropipette data are expressed as mean ±1 SE where sample sizes allow.

pores only if they are not more than 1.5 μm smaller than the cells. The correlation of mean leukocyte diameter with the pore diameter through which 50% of cells traverse was high ($r = 0.73$, $P < 0.01$).

Elastometry. The deformability of leukemic cells in microcapillary tubes was similar to that observed with Nucleopore filters. Although the deformability index at a negative pressure of 50 mm H₂O was slightly greater for leukemic small lymphocytes as compared to leukemic myeloblasts (Table II). The red cell was 3–4 times more deformable than either type of leukemic cell. Leukemic cells would not enter a micropipette with a diameter of 3 μm, a result similar to that observed with filtration. Approximately 50% of leukemic small lymphocytes were able to traverse a micropipette with a diameter of 5 μm, whereas, approximately 50% of leukemic myeloblasts could not enter a micropipette with an orifice diameter of 7 μm, a size which allowed passage of all lymphocytes (Fig. 7).

The optical resolution of a small projection of the outer cell membrane in the micropipette tip is poor. It appears that nuclear size and shape may be a critical factor in the ability to traverse restrictive pores, since at high pressures a tongue of cell enters the pipette leav-
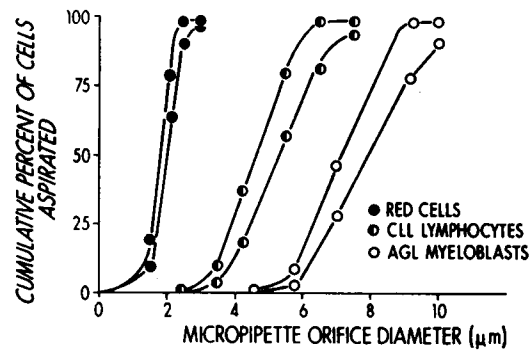

FIGURE 7 The cumulative percent of red cells, leukemia lymphocytes, and myeloblasts able to enter a micropipette with an orificial diameter of 1.7–10 μm at a negative pressure of 50 mm H₂O is shown. The ability to traverse the micropipette was similar to the ability of cells to traverse filters of similar pore size (Fig. 6). The deformability index of red cells was higher when studied using the micropipette as compared to Nucleopore filtration. As shown in Table II by the d_{50}, the ability of each cell type to traverse the micropipette was related to cell diameter ($\bar{d}$).

ing the nucleus surrounded by a very thin cytoplasmic margin (Fig. 8). Leukemic cells have a lower elasticity and higher plasticity than erythrocytes, since when deformed they maintained their disfigurement for a prolonged period before gradually reassuming their approximately spherical shape. These characteristics have been
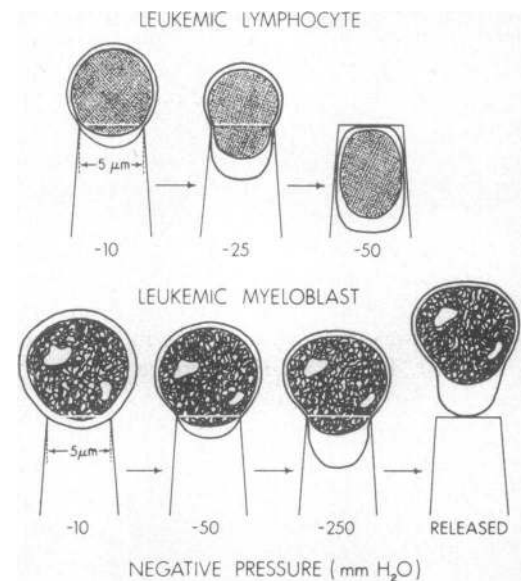

FIGURE 8 A schematic drawing of the shape changes in leukemic lymphocytes and myeloblasts as they are drawn into a 5 μm diameter micropipette at negative pressures up to 250 mm H₂O. Since at higher negative pressures cytoplasm is drawn into the pipette, it appears that the nucleus may be retarding further entry. The lymphocyte nucleus is smaller and it allows passage in the example shown.

previously demonstrated in studies of normal granulo-cytes (18).

DISCUSSION

The relationship of viscosity of leukocyte suspensions to cytocrit is similar to that observed with rigid polystyrene latex spherules. The latter have a viscosity similar to erythrocytes until the packed particle volume reaches 20% after which an exaggerated increase in viscosity occurs as compared to highly deformable particles like erythrocytes (23). Leukocytes are not completely rigid bodies; hence, the increase in viscosity with increasing cytocrit is not strictly analogous to rigid spheres. The viscosity of the latter particles rises more steeply than leukocytes at high cytocrits (20–60%). The increased intrinsic viscosity of leukocytes is a function of a more viscous cytoplasm, a large spheroidal nucleus, and a more rigid periphery. Avian erythrocytes have a slightly increased viscosity as compared to mammalian erythrocytes especially at higher cytocrits. This has been explained, in part by the presence of a nucleus (24). The shear dependency of leukocyte suspensions is independent of plasma proteins and is most likely related to the thixotropic character of internal content and structures.

Our studies found blood viscosity in patients with leukemia and high leukocrits to be slightly higher than that of blood with a similar packed cell volume composed of erythrocytes. The reason for the relatively small effect of large concentrations of leukemic cells is evident from our data. Firstly, the viscosity of leukocyte suspensions of less than 20% leukocrit, is only slightly greater than an equal volume of erythrocytes; secondly, an extraordinary increase in white cell count is required to raise the leukocrit above 20% (that is more than 1×10^6 small lymphocytes or 0.5×10^6 myeloblasts/μl) and this is a rare event; and thirdly, such an increase in leukocrit is accompanied by a slightly greater proportional decrease in erythrocrit resulting in a mean total cytocrit of 40% in CGL, 41% in CLL (see linear regression equations, Fig. 5), and 35% in acute leukemia (see Table I). Hence, a large increase in whole blood viscosity is a rare event in leukemia.

The strong negative association of erythrocrit with leukocrit in chronic leukemia is presumably due to the "encroachment" on erythropoiesis of accumulating granulocytes (CGL) and lymphocytes (CLL) in marrow. The greater decrease in erythrocrit for a given increase in leukocrit in CLL as compared to CGL may be related to the fact that an equivalent cytocrit represents the accumulation of approximately twice the number of lymphocytes as granulocytes.

Previous studies performed with capillary tube viscometers have suggested that a marked elevation of blood viscosity may occur in leukemia (8–10). Increased viscosity

has been most notable in studies of blood from subjects with CGL (8). However, measurements with glass capillary tubes are subject to important artifacts (21, 25) because of the influence of neutrophil and platelet aggregation and cell to glass adhesion on apparent viscosity in vitro. Cell aggregation and adhesion would be most important in CGL blood with a high concentration of platelets and neutrophils, whereas, CLL lymphocytes and AGL myeloblasts are much less aggregable and virtually nonadherent to glass. The recent studies of Steinberg and Charm using a cone-plate viscometer indicated that the viscosity of blood of leukemia patients was similar to that of normal individuals unless large increases in leukocrits occurred and total cytocrits were normal (12). Our findings agree with this conclusion.

However, certain extramedullary effects of leukemia may be the reflection of normal or increased whole blood viscosity in relation to a reduced hemoglobin concentration. A large volume of leukocytes prevents the reduction in blood viscosity and the enhancement in flow which occurs secondary to lowered red cell concentration (14). This may be important since reduced blood viscosity as a result of anemia may act to facilitate tissue perfusion rate and reduce the resistance to cardiac output and thereby, the work of the heart (26–29). Such a suggestion is speculative and is difficult to test experimentally. Subjects with leukemia have an increased red cell 2,3-diphosphoglycerate concentration in response to anemia. This compensatory mechanism could enhance oxygen delivery and this, in effect, would shift the hemoglobin concentration/viscosity ratio toward normal. Inferences as to the clinical effect of alterations in whole blood viscosity must be made cautiously as has been previously emphasized (30, 31).

The alteration in flow due to large numbers of leukocytes may be most consequential in the microcirculation (a) in situations in which cells of large diameter (leukemic blast cells) accumulate and (b) in vessels of internal diameter smaller than that of the cell. The severity of hinderance to flow by leukocyte occlusion in such vessels is not reflected in blood viscosity measurements. In the microcirculation in vessels of capillary and precapillary size, an increase in blood viscosity may be less important than the very high intrinsic viscosity and low deformability of individual leukemic blast cells. These characteristics of leukocytes are better represented by filtration and micropipette studies of individual cells in channels of small diameter.

The inability of immature granulocytes and lymphocytes to traverse narrow channels may also be due to the restriction provided by a poorly deformable spheroidal nucleus. Since leukemic myeloblasts have a cell and nuclear diameter 30–40% greater than lymphocytes and since they are incapable of migrating from the cir-

culation normally because of their inability to adhere, deform, and move efficiently (16, 32), they may be more easily sequestered or wedged in microvascular beds and result in impairment of flow and local injury. Myeloblasts, therefore, may result in leukostasis and vascular damage. This may account for certain instances of hemorrhage and tissue entry of leukemic blast cells. The possible importance of such a sequence of events in the cerebral circulation of leukemic patients had been discussed by Phair, Anderson, and Namiki (33).

Several symptoms of leukemia whose pathogenesis is unexplained, such as joint, bone, and abdominal pain and episodes of tissue infarction, may be a consequence of leukocclusive events. Other cytokinetic features of leukemic cells such as splenic entrapment and prolonged intravascular survival also may be due to rheological properties of these cells which are not conducive to normal transit in the peripheral circulation. Disruption of vascular beds may be the primary route of entry of leukemic cells into tissue. In addition, trapped leukemic blast cells compete with normal tissue for oxygen in stagnant microcirculatory beds. The inability of leukemic cells to adhere and aggregate readily and their relatively low concentrations in blood may reduce the frequency and magnitude of vaso occlusive events in patients with leukemia.

The potential for retardation of blood flow by mature neutrophils has been suggested by studies in hamster cheek pouches (34), rabbit ear chambers (35), and microcapillary tubes (18, 32, 36). The intravascular flow characteristics of animal leukocytes and their possible effect in the circulation have been summarized previously (37). However, normal mature granulocytes are capable of marked attenuation and can traverse pores of small diameters (18, 32).[2] Moreover, they adhere to endothelial cells and emigrate rapidly from the intravascular space (32, 37).

ACKNOWLEDGMENTS

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