Cell Cycle-Dependence of HL-60 Cell Deformability

Mientao A. Tsai, Richard E. Waugh, and Peter C. Keng

Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642 USA

ABSTRACT   In this study, the role of cytoskeleton in HL-60 deformability during the cell cycle was investigated. G_1, S, and G_2/M cell fractions were separated by centrifugal elutriation. Cell deformability was evaluated by pipette aspiration. Tested at the same aspiration pressures, S cells were found to be less deformable than G_1 cells. Moreover, HL-60 cells exhibited power-law fluid behavior: $\mu = \mu_c(\dot{\gamma}_m/\dot{\gamma}_c)^{-b}$, where μ is cytoplasmic viscosity, $\dot{\gamma}_m$ is mean shear rate, μ_c is the characteristic viscosity at the characteristic shear rate $\dot{\gamma}_c$, and b is a material constant. At a given shear rate, S cells ($\mu_c = 276 \pm 14$ Pa·s, $b = 0.51 \pm 0.03$) were more viscous than G_1 cells ($\mu_c = 197 \pm 25$, $b = 0.53 \pm 0.02$). To evaluate the relative importance of different cytoskeletal components in these cell cycle-dependent properties, HL-60 cells were treated with 30 μM dihydrocytochalasin B (DHB) to disrupt F-actin or 100 μM colchicine to collapse microtubules. DHB dramatically softened both G_1 and S cells, which reduced the material constants μ_c by ~65% and b by 20–30%. Colchicine had a limited effect on G_1 cells but significantly reduced μ_c of S cells (~25%). Thus, F-actin plays the predominate role in determining cell mechanical properties, but disruption of microtubules may also influence the behavior of proliferating cells in a cell cycle-dependent fashion.

INTRODUCTION

Leukocytes in the bloodstream are few in number in comparison to red blood cells, but they significantly influence blood flow and oxygen delivery in the microvasculature because of their large volume and low deformability. Increasing evidence has shown that mechanical deformability of leukocytes plays an important role in cell retention in the capillaries (Erzurum et al., 1992; Harris and Skalak, 1993; Worthen et al., 1989), which occurs in ischemia and reperfusion, or before tissue injury. Under pathological conditions, such as in most cases of chronic myelogenous leukemia and acute myeloid leukemia, the circulating concentration of leukocytes is elevated, by as much as 10- to 100-fold (Chervenick and Boggs, 1968; Clarkson and Strife, 1991; Galbraith and Abu-Zahra, 1972; Mauer and Jarrold, 1963), and a large fraction of immature leukocytes (20–50%) may be found in the peripheral blood. Early studies indicate that the immature leukemic cells were markedly more rigid than their mature counterparts (Lichtman and Kearney, 1976). These cells are believed to impair microcirculation to the eye, the central nervous system, or the lungs, hence causing deleterious effects to the organs involved (Lichtman, 1984). Therefore, our understanding of leukocyte deformability is an important aspect for understanding the pathophysiology of such diseases and developing rational therapeutic strategies.

HL-60 is a human leukemic cell line with characteristics similar to those of promyeloblasts and promyelocytes (Col-lins et al., 1977). Since the establishment of the HL-60 cell line, it has been widely used to investigate the cellular and molecular biology of leukocyte proliferation and differentiation (Collins, 1987). In this study, we used the HL-60 cell as a model system to characterize the mechanical behavior of immature leukemic leukocytes and to assess their potential to impair the microcirculation. In particular, we quantitatively measured the deformability of HL-60 cells and the influence of the cytoskeleton in cellular mechanical properties during cell proliferation. Ultimately this study will give us a fundamental understanding of how leukocytes behave in the microvasculature, and shed new light on how the mechanical properties of leukocytes are regulated by the cellular structure.

MATERIALS AND METHODS

Cell culture

The HL-60 cell line was obtained from the American Type Culture Collection (Bethesda, MD). Cells were grown in a suspension of RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT) and maintained at 37°C with 5% CO_2 and 100% humidity. Cells were grown at an initial concentration of 2–3 × 10^5 cells/ml and passaged twice each week. HL-60 cells in the exponential growth phase were fractionated by centrifugal elutriation and subjected to micromechanical measurements.

Cell elutriation

The procedures for obtaining relatively homogeneous populations of G_0/G_1, S, and G_2/M cells by centrifugal elutriation have been described in detail previously (Keng et al., 1980; Palis et al., 1988). Briefly, 1–2 × 10^8 HL-60 cells in exponential growth were centrifuged and collected into 20 ml of fresh RPMI 1640, then loaded into the Sanderson separation chamber of the Beckman JE6 elutriator system (Beckman Instruments, Palo Alto, CA) at a rotor speed of 3200 rpm and a fluid flow rate of 32 ml/min. After a 200-ml sample was collected, the rotor speed was decreased in small steps (50–200 rpm) to 2000 rpm and a series of 50-ml fractions were

collected at each rotor speed. The separation procedures were performed under sterile conditions at 4°C to minimize the cell cycle progression. Cells in each fraction were counted and their volume distributions were measured immediately using a Coulter counter and channelyzer system (model c1000; Coulter Electronics, Hialeah, FL).

Flow cytometry

The homogeneity of each elutriated fraction was analyzed by flow cytometry. About 1×10^6 cells from each fraction were fixed in 3 ml of 75% ethyl alcohol and stored at 4°C over 24 h. These cells were then incubated in 1 ml of 1 mg/ml RNase at room temperature for 30 min and stained with 1 ml of 10 μg/ml propidium iodide (Molecular Probes, Eugene, OR) for DNA analysis on an Epics Profile flow cytometer (Coulter Electronics). DNA histograms were analyzed according to the mathematical model of Fried et al. (1976). The relationship between cell volume and cell cycle positions was determined according to the method of Brosing et al. (1986).

Colchicine and DHB treatments

HL-60 cells were treated either with 100 μM colchicine to collapse the microtubules or with 30 μM dihydrocytochalasin B (DHB) to disrupt F-actin. Colchicine and DHB were purchased from Sigma Chemical (St. Louis, MO). DHB was dissolved in dimethyl sulfoxide into 6 mM stock solution and was kept frozen at −20°C. Colchicine was dissolved in distilled water into 100 mM stock and kept at 4°C. When used for experiments, the stock solutions were diluted with fresh phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS) (Whittaker Bioproducts, Walkersville, MD) to twice the desired concentrations. A 1:1 (v/v) dilution with the cell suspension yielded the desired drug concentrations. Micromechanical measurements were made 15 min after cells were exposed to the reagents and were finished within 90 min.

Micromechanical measurements

Details of cell micromanipulation and micromechanical measurements have been described elsewhere (Tsai et al., 1993). Briefly, HL-60 cells were suspended in PBS at a concentration of 5×10^5 cells/ml and supplemented with 5% FBS. The suspension was placed in a U-shaped microchamber with a cavity of 10 mm × 20 mm × 3 mm. A micropipette was inserted from the open side into the chamber, and a negative aspiration pressure was generated by displacing a hydraulic reservoir. The pressure was measured to an accuracy of 5 Pa (0.05 cm H_2O). Single cells in their resting (passive) state were chosen and aspirated into micropipettes at a series of fixed suction pressures ranging from 0.3 to 1.5 kPa (3.0–15.0 cm H_2O). Different-sized pipettes were used so that the geometry of deformation (i.e., the ratio between cell and pipette radii) was the same for all cell subpopulations (Fig. 1). HL-60 cytoplasmic viscosity and the mean shear

rate during cell entry were calculated based on a numerical analysis of the cell entry into a micropipette (Tsai et al., 1993).

RESULTS

Cell elutriation and flow cytometry

Exponentially growing HL-60 cells were separated, as described in Materials and Methods, into fractions based on their difference in cell size. Shown in Fig. 2 is the typical cell volume distribution of three populations of HL-60 cells (G_1, S, G_2/M) separated by centrifugal elutriation. DNA histograms for these cell populations were analyzed by flow cytometry. The results are shown in Table 1. The results indicate that enriched G_1 (>95%) and S (>75%) cells can be obtained by the centrifugal elutriation. These cell subpopulations were used to make micromechanical measurements. During the micromechanical experiments on the "S" cell fraction, cells with large volumes were chosen on a single-cell basis to improve the likelihood that the sampled cell population consisted exclusively of cells in the S phase.

Cell cycle dependence of HL-60 cell deformability

Shown in Fig. 3 and Table 2 are results from a typical micromechanical experiment of HL-60 cells in G_1 and S phases when tested at a series of aspiration pressures ranging from 0.5 to 1.3 kPa. In this particular experiment, cytoplasmic viscosity of HL-60 cells was found to range from 350 to 127 Pa·s in S phase and from 207 to 71 Pa·s in G_1 phase. In some other experiments, HL-60 cytoplasmic viscosity was measured as high as 500 Pa·s for cells in the S phase when tested at a lower aspiration pressure, 0.3 kPa. It is shown clearly that the mechanical properties of HL-60 cells are cell cycle dependent. HL-60 cells in S phase are markedly more viscous than those in the G_1 phase of the cell cycle when compared at the same aspiration pressures. A pooled t-test was performed using DataDesk (Odesta, Northbrook, IL). A significant difference was found between G_1 and S populations when compared at the same aspiration pressures (Table 2). G_2/M cells in turn were found to be much more viscous than the S cells. The

FIGURE 1   Video micrograph of HL-60 cells in G_1 and S phases entering different-sized micropipettes in response to a stepwise aspiration pressure. (a–d) Video sequence of a G_1 cell entering a 6.5-μm pipette; (e–h) video sequence of an S cell entering a 7.3-μm pipette. The ratios of cell diameter to pipette size for G_1 and S cells are the same, ~1.75. Magnification is the same for all figures. Bar = 10 μm.

The header shows Tsai et al., Cell Cycle Dependence of HL-60 Cell Deformability, 2025
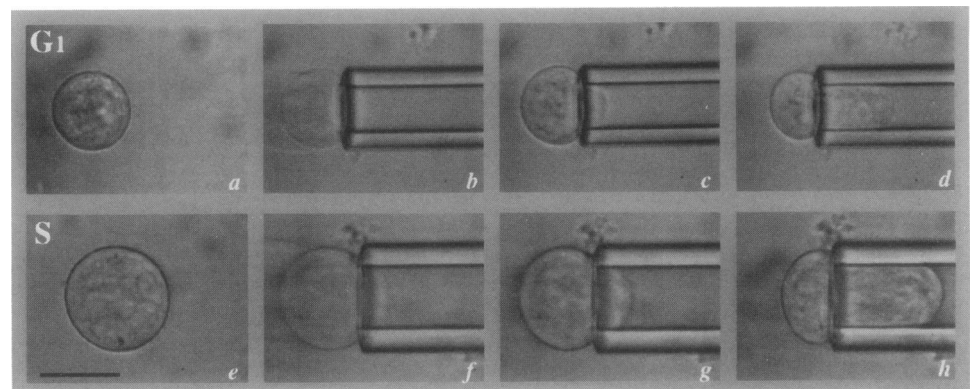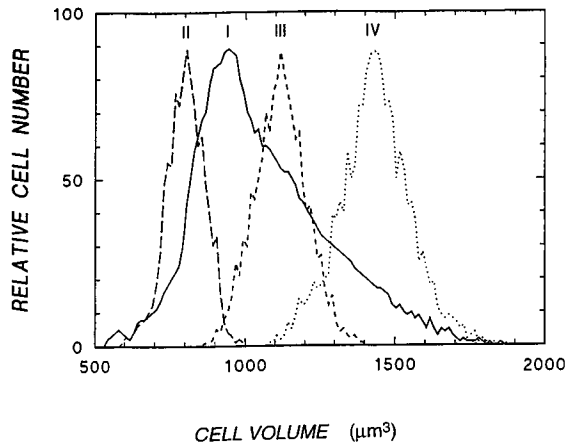
FIGURE 2 The cell volume distribution of HL-60 cells measured by Coulter counter and channelyzer system. (*I*) HL-60 cells before separation by centrifugal elutriation. (*II*, *III*, *IV*) the three populations (G$_1$, S, and G$_2$/M) of HL-60 cells selected from the fractions obtained by centrifugal elutriation of the cells shown in I. The average volume of G$_1$ cells is 796 μm^3 (11.5 μm in diameter) and the mean volume of S cells is 1150 μm^3 (13.0 μm).
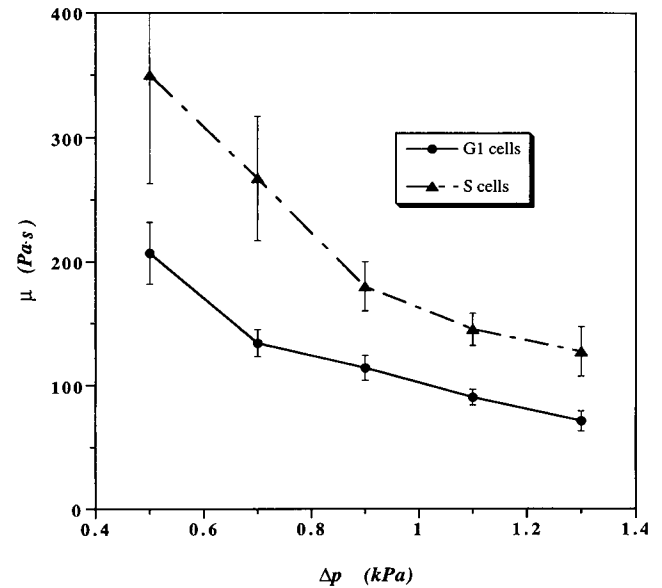

FIGURE 3 HL-60 cell cytoplasmic viscosity as a function of aspiration pressure. The apparent viscosity varies from 350 to 127 Pa·s for S cells and 207 to 71 Pa·s for G$_1$ cells when tested at a series of aspiration pressures ranging from 0.5 to 1.3 kPa (5.0 to 13.0 cm H$_2$O). It is shown that HL-60 cells in G$_1$ phase are more deformable than those in S phase when tested at the same aspiration pressure.

cytoplasmic viscosity of G$_2$/M cells was found to be 10- to 100-fold greater than that of S cells at the same aspiration pressures. For instance, the viscosity of G$_2$ cells was measured to be 4010 ± 756 Pa·s ($n = 13$) at an aspiration pressure of 0.9 kPa. Frequently, the G$_2$/M cells could not complete the entry into the pipette at all. Observed under the microscope, it appeared that cell entry was limited by availability of excess surface area of the cell membrane. There was no evidence of adhesion between the cells and the pipette when a non-entering cell was released from the pipette, and when very large aspiration pressures were applied to some cells, lysis was observed. Because of these difficulties, we focused our effort on the differences between G$_1$ and S cells.

Shear thinning of cytoplasmic viscosity

It is also evident in Fig. 3 and Table 2 that HL-60 cytoplasmic viscosity depends strongly on the aspiration pressure, that is, the cytoplasm behaves as a non-Newtonian fluid. Shown in Fig. 4 is the cytoplasmic viscosity as a function of the mean shear rate during cell entry in logarithmic scale. Both groups of cells exhibited shear-thinning behavior, i.e., the apparent viscosity decreased with increasing shear rate, as has been observed for normal neutrophils. The shear rate

dependence of the cytoplasmic viscosity can be approximated as that of a power-law fluid (Tsai et al., 1993):

$$\mu = \mu_c(\dot{\gamma}_m/\dot{\gamma}_c)^{-b},$$

where μ is the cytoplasmic viscosity, $\dot{\gamma}_m$ is mean shear rate during cell entry, μ_c is the characteristic viscosity at the characteristic shear rate $\dot{\gamma}_c$, and b is a material coefficient. The material constants for HL-60 cells in G$_1$ and S phases of the cell cycle are summarized in Table 3. When $\dot{\gamma}_c$ is set to 1 s^{-1}, $\mu_c = 197 ± 25$ Pa·s and $b = 0.53 ± 0.02$ for G$_1$ cells, and $\mu_c = 276 ± 14$ Pa·s and $b = 0.51 ± 0.03$ for S cells. These results indicate that at the same shear rate, HL-60 cells in the S phase of the cell cycle are more viscous than those in G$_1$ phase. When compared at the same shear rate, HL-60 cells, even in G$_1$ phase, are less deformable than normal neutrophils ($\mu_c = 130 ± 23$ Pa·s, $b = 0.52 ± 0.09$) (Tsai et al., 1993).

TABLE 1 Cell cycle distribution of HL-60 cells based on DNA histogram analysis by flow cytometry

Cell population	G$_1$ cells (%)	S cells (%)	G$_2$/M cells (%)
Unseparated	58	29	13
G$_1$ enriched	96	4	0
S enriched	24	76	0

TABLE 2 A comparison between values of G$_1$ and S cell cytoplasmic viscosity when tested at a series of aspiration pressures

Aspiration pressure Δp (kPa)	Apparent viscosity μ (Pa · s)		Pooled t-test
	G$_1$ cells	S cells	
0.5	207 ± 25	350 ± 87	$p < 0.05$
0.7	134 ± 11	267 ± 50	$p < 0.01$
0.9	114 ± 10	180 ± 20	$p < 0.01$
1.1	90 ± 6	145 ± 13	$p < 0.01$
1.3	71 ± 8	127 ± 20	$p < 0.01$

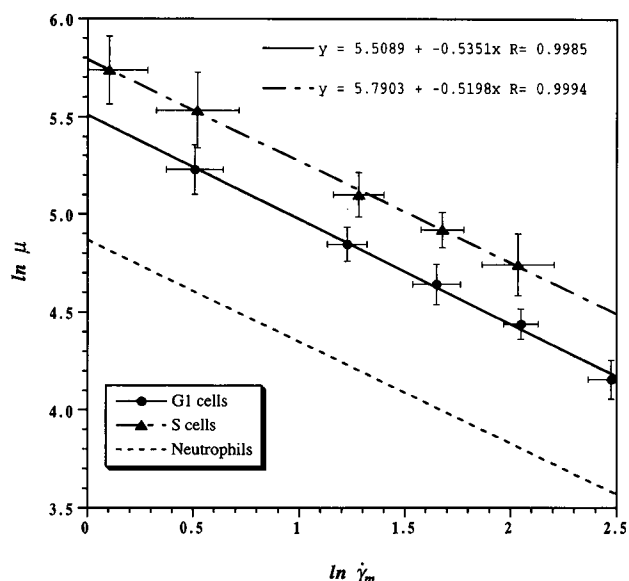The ± indicates standard errors ($n = 15$–25).

FIGURE 4 HL-60 cytoplasmic viscosity as a function of the mean shear rate in logarithmic scale. Each data point represents the mean of 15–20 cells at a given aspiration pressure, and the vertical and horizontal bars are the SDs. Generally, HL-60 cytoplasmic viscosity and the mean shear rate follow a power-law relationship. It is evident that at a given shear rate, S cells (*dotted-dash line with closed triangles*) are more viscous than G_1 cells (*solid line with closed circles*), which in turn are more viscous than their mature counterpart, the neutrophil (*dashed line*).

Effect of DHB and colchicine

To gain insight into the role that different components of the cytoskeleton play in the cell cycle dependence of HL-60 cell mechanical properties, G_1 and S cells were treated with 30 μM dihydrocytochalasin B (DHB) to disrupt F-actin (microfilaments) or 100 μM colchicine to depolymerize the microtubules. Shown in Fig. 5 are the effects of colchicine and DHB on cytoplasmic viscosities of G_1 and S cells from a typical experiment. Summarized in Tables 4 and 5 are the results from triplicate experiments. When compared at the same aspiration pressures, both reagents significantly softened HL-60 cells at both G_1 and S phases of the cell cycle (Fig. 5). DHB had a more dramatic effect than colchicine. DHB reduced the cytoplasmic viscosity of both G_1 and S cells by as much as 60–85% (Tables 4 and 5). It is interesting that colchicine had different effects on G_1 and S cells, which reduced the cytoplasmic viscosity of S cells by 35–
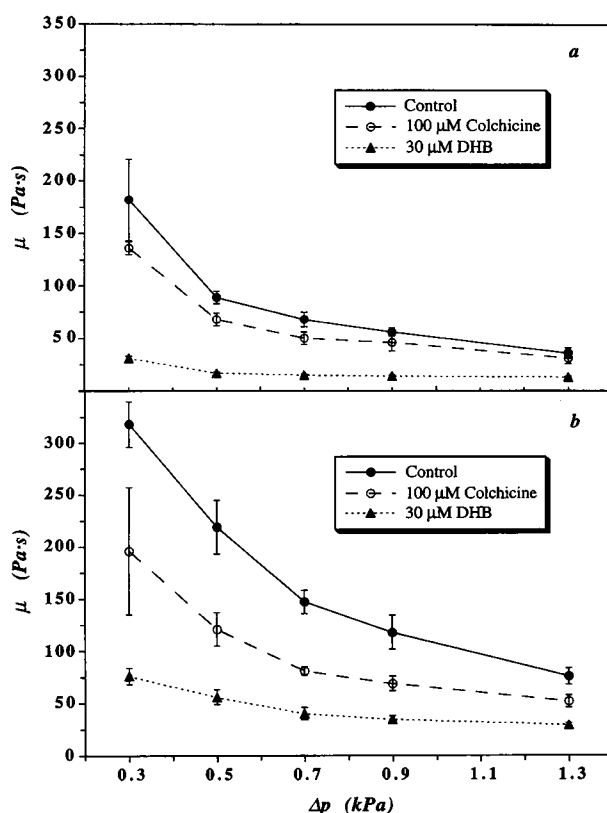

FIGURE 5 Effect of colchicine and dihydrocytochalasin B on cytoplasmic viscosity of G_1 (*a*) and S (*b*) HL-60 cells at different aspiration pressures. The solid line with filled circles represents untreated cells. The long dashed line with open circles shows cells treated with 100 μM colchicine. The dotted line with closed triangles indicates cells treated with 30 μM DHB. Both reagents significantly softened G_1 and S cells, compared at the same aspiration pressure.

45% (Table 5), but reduced the viscosity of G_1 cells only by 20–25% (Table 4).

The effects of colchicine and DHB on the shear rate dependence of HL-60 cells are shown in Fig. 6. The results from the triplicate experiments are summarized in Table 6. DHB dramatically reduced the characteristic viscosity of both G_1 and S cells by 60–65%. It also significantly decreased the shear rate dependence of both G_1 (~29%) and S cells (~17%). Colchicine, on the hand, had a very limited effect on G_1 cells, but had a significant effect on the characteristic viscosity of S cells (~25%), without a significant effect on their shear rate dependence (Table 6).

DISCUSSION

Cell cycle dependence of cell mechanical properties

Our results show that mechanical properties of proliferating HL-60 cells were dependent on their position in the cell cycle. These results are consistent with earlier reports by Anderson et al. (1991) and Needham et al. (1991), who documented that growing populations of hybridoma and

TABLE 3 Material constants of HL-60 cells and neutrophils based on the power-law fluid model

Cell group	μ_c (Pa · s)*	b
HL-60 S cells	276 ± 14	0.51 ± 0.03
HL-60 G_1 cells	197 ± 25	0.53 ± 0.02
Neutrophils[‡]	130 ± 23	0.52 ± 0.09

Mean ± SD (n = 11).

*The value of μ_c was determined when $\dot{\gamma}_c$ = 1 s^{-1}.

[‡]Data were taken from Tsai et al. (1993).

TABLE 4 Reduction of cytoplasmic viscosity by colchicine and DHB on G$_1$ HL-60 cells at different aspiration pressures, averaged from triplicate experiments

Δp (kPa)	Control G$_1$ cells		100 μM colchicine		30 μM DHB	
	μ (Pa · s)	Δ (%)	μ (Pa · s)	Δ (%)	μ (Pa · s)	Δ (%)
0.3	182 ± 99	—	115 ± 34	−26 ± 1	37 ± 25	−84 ± 2
0.5	103 ± 60	—	65 ± 17	−22 ± 3	27 ± 20	−76 ± 5
0.7	78 ± 40	—	50 ± 25	−25 ± 6	21 ± 17	−73 ± 7
0.9	55 ± 33	—	41 ± 28	−22 ± 3	21 ± 14	−65 ± 8
1.3	39 ± 24	—	28 ± 17	−2 ± 5	17 ± 11	−59 ± 6

Mean ± SD.

transformed fibroblast cell lines were heterogeneous in size and morphology and that their rheological properties were cell cycle dependent. These earlier studies relied on morphological criteria to select cells in different stages of the cell cycle, whereas, in the present report, separate populations of cells were generated by centrifugal elutriation and the stage in the cell cycle was verified by direct measurement of DNA content. In spite of these different approaches for identifying cells in a specific stage of the cell cycle and differences in the types of cells tested, all of the studies indicate that cells in S and G$_2$/M phases are intrinsically more viscous than cells in the G$_1$ phase.

It has been well documented in our earlier work (Tsai et al., 1993, 1994) as well as in the current study that cellular mechanical properties exhibit a strong dependence on deformation rate. Hochmuth and colleagues (1993) have indicated that cellular properties may also depend on the magnitude of deformation. These dependencies on cell size and rate of deformation are particularly important when dealing with heterogeneous cell populations, because differences in the calculated viscosity that may result from differences in the geometry of the cells might be erroneously interpreted as differences in the intrinsic properties of the cells. To avoid such complications, different-sized pipettes were used so that the ratio of the mean cell diameter to the pipette diameter was the same for both small (G$_1$) and large (S) cells, and the measurements were performed over a range of aspiration pressures so that the rate dependence of the cellular behavior could be assessed. Thus, the differences in properties that we have observed reflect true differences in intrinsic deformability and are not a consequence of different conditions of measurement. It is also important to recognize that the shear rate dependence of the cytoplasmic

viscosity that has been observed is a real property of the cells and not a consequence of the particular method of calculation that was employed (Tsai et al., 1993). Although the method of calculation does involve some approximations, this approach avoids overestimation of the difference in properties between slowly deforming and rapidly deforming cells that might otherwise occur.

Cytoskeleton and cell mechanical properties

Consistent with our previous work (Tsai et al., 1994), our results show that cytoskeletally associated microfilaments (F-actin) play the major role in determining the mechanical properties of leukocytes. Weakening of the F-actin network with 30 μM dihydrocytochalasin B dramatically reduces the cytoplasmic viscosity of the proliferating HL-60 cells. This finding is in good agreement with the results of cell poker indentation experiments (Erzurum et al., 1991), in which it was shown that disruption of F-actin can reduce HL-60 cell relative rigidity by ~67% (from 0.206 to 0.067 mdyne/μm). In addition to documenting the important role of actin filaments, the present study provides the first evidence that the mechanical properties of the proliferating HL-60 cells may be significantly influenced by the microtubules, and furthermore, the effect of disrupting microtubules is cell cycle dependent.

It has been reported in MRC-5 fibroblasts that the amount of F-actin and tubulin in cells doubles from G$_1$ to G$_2$ phases during the cell cycle (Leger et al., 1990). Inasmuch as the cell volume also doubles as the cells grow from G$_1$ to G$_2$, it is not expected this increase in content would necessarily result in an increase in rigidity, because the concentration of

TABLE 5 Reduction of cytoplasmic viscosity by colchicine and DHB on S HL-60 cells at different aspiration pressures, averaged from triplicate experiments

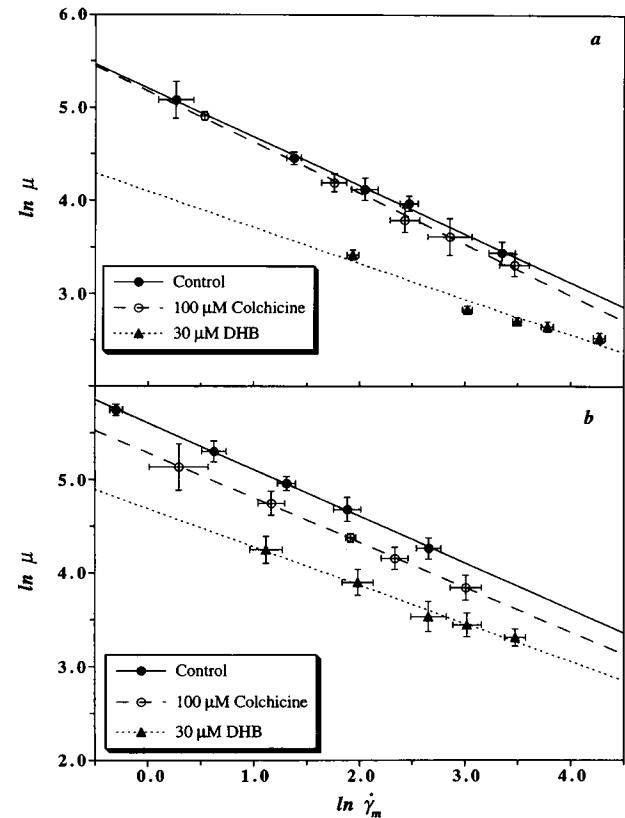| Δp (kPa)	Control S cells		100 μM colchicine		30 μM DHB	
	μ (Pa · s)	Δ (%)	μ (Pa · s)	Δ (%)	μ (Pa · s)	Δ (%)
0.3	318 ± 64	—	192 ± 155	−39 ± 1	71 ± 27	−77 ± 1
0.5	221 ± 88	—	115 ± 64	−44 ± 1	51 ± 29	−77 ± 4
0.7	146 ± 57	—	86 ± 36	−38 ± 6	37 ± 17	−75 ± 4
0.9	108 ± 48	—	67 ± 26	−39 ± 3	33 ± 18	−70 ± 6
1.3	75 ± 26	—	46 ± 18	−37 ± 6	25 ± 7	−67 ± 5

Mean ± SD.

FIGURE 6 Effect of colchicine and DHB on G_1 (a) and S (b) HL-60 cytoplasmic viscosity as a function of the mean shear rate during cell entry. The solid line with closed circles represents untreated cells. The long dashed line with open circles shows cells treated with 100 μM colchicine. The dotted line with closed triangles indicates cells treated with 30 μM DHB. DHB significantly reduces both characteristic viscosity and shear rate dependence of G_1 and S cells. Colchicine, on the other hand, has a slight effect on G_1 cells, but significantly reduces the characteristic viscosity of S cells without any change in their dependence on shear rate.

actin filaments and microtubules remains relatively constant. Thus, in addition to an increase in the amount of cytoskeletal proteins, the increase in rigidity that we have observed indicates that there must also be changes in the organization and cross-linking of these proteins. It is also possible that synthesis of DNA and reorganization of the nucleus may contribute to the cell cycle dependence of cellular mechanical properties. Further studies are needed to investigate the precise nature of the changes in the cytoskel-

eton and other structural components of the cell that account for the changes in cellular properties that we have observed.

Cellular deformability and leukocyte trafficking

It has long been thought that cellular deformability is one of the factors that regulate leukocyte release from the bone marrow through the endothelial sinus pores (Lichtman et al., 1977, 1989). It has been shown that when leukemic cells were arrested in S, G_2, or M phase by cell cycle-specific drugs, their emission from the marrow was inhibited (Stryckmans et al., 1977). It was suggested that the G_1 cells are smaller and hence enter the blood more easily (Baccarani and Killmann, 1972; Killmann et al., 1963). The present results indicate that leukocytes in the S or G_2/M phase not only are larger in size, but also are intrinsically more rigid than the cells in G_1 phase. This effect is further amplified by the fact that leukocyte cytoplasmic viscosity is shear thinning, i.e., the more slowly leukocytes deform, the more viscous the leukocyte cytoplasm is. These findings provide further support for the view that the deformability of leukocytes may serve as a factor that controls cell egress from the bone marrow.

In the microcirculation, the diameter of the capillaries is often considerably smaller than that of leukocytes (Folkow and Neil, 1972). Thus, leukocytes must deform into a sausage shape to traverse the capillaries. It has been shown in vitro by Erzurum et al. (1991) that the stiffer the cells are, the more the cells are trapped in capillary-sized micropores. Under pathological conditions, such as in some cases of human leukemia, immature leukocytes accumulate in the blood in extraordinary concentration. These immature cells are poorly deformable in comparison with their mature counterparts (Fig. 4 and Table 3). This would contribute to the obstruction of the flow in the microvasculature, as clinically observed in hyperleukocytic leukemia (Lichtman and Rowe, 1982). The present data also indicate that the immature leukocytes at S or G_2/M phase in the cell cycle would have a much higher risk of impairing flow in the microvasculature should they reach the peripheral circulation.

CONCLUSION

Our results show that cellular deformability of proliferating HL-60 cells depends on the cell cycle, and that cells in the

TABLE 6 Effects of colchicine and DHB on the material constant of G_1 and S HL-60 cells, based on the power-law fluid model, where $\dot{\gamma}_c = 1$ s^{-1}

Drug treatment	G_1 cells				S cells			
	μ_c (Pa·s)	Δ (%)	b	Δ (%)	μ_c (Pa·s)	Δ (%)	b	Δ (%)
Control	197 ± 25	—	0.53 ± 0.02	—	276 ± 14	—	0.51 ± 0.03	—
Colchicine	185 ± 27	−6 ± 3	0.54 ± 0.03	NS*	209 ± 22	−24 ± 4	0.51 ± 0.03	NS*
DHB	73 ± 34	−64 ± 12	0.38 ± 0.03	−29 ± 8	105 ± 7	−62 ± 4	0.42 ± 0.02	−17 ± 9

Mean ± SD ($n = 3$).
*Not significant.

G_1 phase are intrinsically more deformable than those in the S phase, which in turn are more deformable than cells in the G_2/M phase. This study also supports the view that F-actin plays the predominant role in determining leukocyte mechanical properties. Furthermore, it provides the first evidence that microtubules may also have a significant influence on the mechanical properties of proliferating cells and that the contribution from microtubules depends on the cell cycle.

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