Human Lymphocyte Motility: Normal Characteristics and Anomalous Behavior of Chronic Lymphocytic Leukemia Cells

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The characteristics of human lymphocyte motility and its relationship to the redistribution of surface membrane antigens (capping) are poorly defined. Since chronic lymphocytic leukemia (CLL) cells cap poorly when compared with normal human lymphocytes, this study was undertaken to compare the motility of these two cell types. A modification of the Boyden chamber system was employed to quantify lymphocyte motility by placing lymphocyte suspensions on 8-μm convoluted-pore nitrocellulose filters and measuring the depth of migration of the cells into the filter at 37°C. After 3 hr of incubation, CLL cells migrated significantly less into the filter than normal cells. Incubation in the presence of sodium azide or at 4°C abolished all motility, indicating the active nature of the process. The relative motility of individual CLL patients' cells correlated best with the proportion of abnormal cells present as determined by surface receptor assays. The possibility that decreased cell motility in CLL was a reflection of enrichment by a "bone marrow-derived" (B cell) population was eliminated by the finding that normal B cells purified by gradient separation of rosetted cells migrated faster than normal T cells and considerably faster than CLL cells. Motility of normal and CLL lymphocytes was decreased by cytochalasin B and increased by colchicine, vincristine, and vinblastine. Thus, human lymphocyte motility appears to be dependent on microfilament integrity but not to require the colchicine-sensitive cytoskeleton. The decreased motility of CLL cells is the result of an intrinsic cell abnormality, but this finding cannot fully explain the decreased capping, since in human lymphocytes the latter is not prevented by an inhibitor of motility.

STUDIES DEMONSTRATING that predominantly motile cell types exhibit redistribution to cell surface components following the binding of certain materials to the cell membrane (capping) have suggested that this process might be related to cell movement or locomotion. One type of motile cell that has been shown to exhibit the capping phenomenon is the normal human peripheral blood lymphocyte. In contrast, we have previously demonstrated that lymphocytes from the peripheral blood of patients with chronic lymphocytic leukemia (CLL)* do not cap normally. Thus, it was of importance...
to determine whether CLL lymphocytes possess an altered pattern of cell movement that might correlate with this decreased surface antigen redistribution.

Previous studies of lymphocyte motility have employed semiquantitative assays of locomotion involving the measurement of the distance moved by lymphocytes along a glass slide during several days in culture or even more indirect methods involving the counting of the percentage of lymphocytes exhibiting a morphological configuration which has been associated with cell movement. To quantify better lymphocyte motility in vitro, we modified the Boyden chamber technique which has previously been used to study the migration of polymorphonuclear leukocytes and macrophages. This methodology allowed examination of the locomotion of normal human lymphocytes and CLL lymphocytes by measuring the depth of cell migration into micropore filters during short-term incubations. In addition, the migration of normal lymphocyte subpopulations and the effects of inhibitors, felt to affect predominantly microfilaments and microtubules, upon motility were studied. The methods employed have allowed further characterization of the locomotion of normal lymphocytes in a systematic and quantitative manner and the assessment of the migratory capacities of CLL lymphocytes.

MATERIALS AND METHODS

Twenty-five unselected patients with chronic lymphocytic leukemia (mean age 58 yr) from the Durham Veterans Administration and Duke Hospitals were studied. Some of these patients were untreated and others were under treatment at the time of the study, but all had peripheral blood lymphocyte abnormalities. Control subjects consisted of two healthy subgroups, the first of which was a group of 11 medical students and laboratory technicians with a mean age of 28 yr. The second subgroup consisted of nine older patients (mean age 60 yr) from the Durham VA Hospital Orthopedic ward. These patients were hospitalized for prosthetic fittings or were recovering from fractures of an extremity. All subjects consented to have blood drawn for the study.

Lymphocyte Purification

Unless otherwise stated, plastic materials (Falcon Plastics, Oxnard, Calif.) were used for all studies involving cell contact. From each subject, 20-60 cc of heparinized blood was obtained, mixed with plasmagel (HTI Corp., Buffalo, N.Y.), (10 cc plasmagel/25 cc blood), and allowed to settle at room temperature for 20-30 min and purified as previously reported. The leukocyte-rich supernatant was removed, mixed with carbonyl iron powder (GAF Corp., Atlanta, Ga.) in a plastic beaker, and the mixture was shaken gently for 20 min in a 37°C water bath. Following incubation, a magnet was used to trap the iron and phagocytic cells at the bottom of the beaker, and the lymphocyte-rich supernatant was removed. Final purification of lymphocytes was achieved by placing this supernatant on a Ficoll-Hypaque gradient (a 3:1 mixture of 9 Ficoll [Pharmacia Fine Chemicals, Piscataway, N.J.] and 50% hypaque [Winthrop Labs., N.Y.]) and centrifuging at 800 g for 30 min. The cells located at the plasma-Ficoll interface were then aspirated, washed once with 0.15-M saline and twice with phosphate-buffered (pH 7.2) saline containing 0.1%, gelatin, and suspended in tissue culture medium 199 (M199) (Grand Island Biological Co., Grand Island, N.Y.). Cells thus purified were composed of at least 98%, lymphocytes, and more than 95%, of them were viable as determined by Trypan blue exclusion. The cells were then diluted with M199 to a concentration of 8 x 10⁶/ml and further diluted with Gey’s solution (Grand Island Biological Co., Grand Island, N.Y.), to a standard final concentration of 2 x 10⁶ cells/ml.

Locomotion Assay

Cell locomotion or motility was assayed using modified Boyden chambers, with 8-μm pore nitrocellulose filters (Brinkmann Instrument Corp., Westbury, N.Y.) used to separate the upper and lower compartments of the chamber. These filters contained convoluted pores, which did
not permit materials to drop straight through, but which required migration in at least two planes. The cells to be assayed (0.4 ml at 2 x 10^6 cells/ml) were placed at the top of the filter, and the compartment below the filter was filled with 0.85 ml buffered solution (0.5 ml Gey’s solution plus 0.35 ml Veronal buffer with 0.1% gelatin at pH 7.5 (GVB)). Incubations were carried out for 3 hr and 18 hr at both 37°C and 4°C in humidified air containing 5% CO2. Following incubation, the fluid from the upper and lower compartments was aspirated simultaneously and the filters removed. The latter were then stained with hematoxylin for 6 min, dehydrated, and cleared with ethanol and xylene and examined using a 40x objective. The depth of migration of cells into the filters was determined by using a microscope with an optical micrometer (Bausch and Lomb Co., Rochester, N.Y.) and measuring the distance from the top of the filter to the plane of focus containing the second furthest migrating cell. Ten separate fields across each filter were scored in this manner. Duplicate chambers were run in all instances, and the mean of all 20 determinations was taken as the index of motility.

Separation of Lymphocyte Populations

As previously reported by Wybran and Fudenberg, if human lymphocytes are allowed to form rosettes with red blood cells (either sheep red blood cells or complement-coated red cells) and then centrifuged on Ficoll-Hypaque gradients, lymphocytes, having formed rosettes, form the pellet in the gradient, while nonrosetted lymphocytes remain at the plasma-Ficoll interface. Therefore lymphocyte subpopulations were prepared by two variations of rosette gradient techniques as follows.

(1) E rosettes (sheep red blood cell rosettes). Four to five milliliters of sheep red blood cells (SRBC) were washed twice with Veronal-buffered saline with gelatin (GVB) and adjusted to a final cell concentration of 1 x 10^7/ml. Two milliliters of SRBC were then added to 2 ml of purified lymphocyte suspension (2 x 10^6/ml). The mixture was centrifuged for 10 min at 200 g and incubated at room temperature for 1 hr. The tubes were then gently shaken to resuspend the cells and an aliquot was taken to determine the percentage of rosette-forming lymphocytes by counting the percentage of cells with three or more red blood cells attached, and the remainder of the mixture was placed on a Ficoll-Hypaque gradient (see above) and centrifuged for 30 min at 400 g. The plasma-Ficoll interface was removed, the cells were washed with saline and then with M199, and adjusted to 2 x 10^6 cells/ml with M199 and Gey’s solution as described above. The population of cells obtained was shown to contain greater than 90%, surface immunoglobulin-containing cells by fluorescent antiimmunoglobulin assay as previously described, and thus was considered to be an enriched B cell population. The cell button in the Ficoll-Hypaque gradient was shown to be depleted of immunoglobulin-containing cells and was considered to be an enriched T-cell population.

(2) HEC3 rosettes. Four to five milliliters of human group O red cells (HE) were washed and processed in a manner similar to that for the sheep red cells, except that the final concentration was adjusted to 4 x 10^6/ml. These cells were then coated with C3 by the addition of normal human serum, cold agglutinin antibody (kindly supplied by Dr. Wendell Rosse), and GVB under conditions resulting in cells coated with C3, but no antibody as previously reported. The HEC3 were then adjusted to 1 x 10^8 cells/ml. Two milliliters of the suspension was then added to 2 ml of purified lymphocyte suspension. The mixture was shaken in a 37°C water bath for 30 min and centrifuged on a Ficoll-Hypaque gradient as described above. The nonrosetted cells located at the plasma-Ficoll interface were then removed, washed as described above, and adjusted to 2 x 10^6 cells/ml. This cell population was shown to be partially, but not completely, depleted of surface immunoglobulin-containing cells (generally containing less than 10%), and was considered as an enriched T-cell population.

Motility of Lymphocyte Subpopulations

Motility of the enriched lymphocyte populations obtained as described above was determined by the assay using 8-μm pore nitrocellulose filters in the Boyden chamber. In order to compare the separated lymphocyte populations with normal unseparated populations under similar conditions, it was necessary to pass purified samples of lymphocytes through a second Ficoll-hypaque gradient and to recover the plasma-Ficoll interface. The purified lymphocytes thus obtained were run as controls to compare with the enriched B- and T-lymphocyte populations. The numbers...
obtained were not directly comparable with the numbers obtained for the purified lymphocytes assayed under the routine conditions, since the former cells spent a longer period of time during processing and were also passed through an additional Ficoll gradient. In addition, because of the small numbers of cells obtained, in order to adjust to the required final cell concentration, these special preparations were adjusted to final concentration in either M 199 alone or M 199 and Gey's solution in a 1:1 dilution. Thus, the nutrient media differed somewhat from the routine motility studies as well. Nevertheless, viability studies of both the separated lymphocyte populations and the total lymphocyte populations with the additional processing revealed greater than 95%, viability at times comparable to those of the initiation of the motility study.

Effects of Chemical Agents Upon Motility

In order to determine the effects of certain agents on lymphocyte motility, media were prepared containing the desired concentration of vincristine sulfate and vinblastine sulfate (Eli Lilly and Co., Indianapolis, Ind.), colchicine (Sigma Chemical Corp., St. Louis, Mo.), sodium azide (Fisher Scientific Co., Fair Lawn, N.J.), and cytochalasin B (Sigma Chemical Corp., St. Louis, Mo.). In the case of all agents except cytochalasin B, the material was dissolved directly in media used for motility determination as described above. Cytochalasin B was first dissolved in 23%, ethanol at a concentration of 190 μg/ml and then diluted with Gey's medium to give a final cytochalasin B concentration of 10 μg/ml. Control media consisting of 2% ethanol in Gey's medium were also prepared. In each instance the locomotion assay was then performed as described above, using the modified Boyden chamber with simultaneous determinations of motility of a given preparation of cells in normal medium and medium containing the agent to be tested. Statistical analysis was performed using Student's t test for paired and unpaired variables.

RESULTS

The cells isolated from the peripheral blood of normal subjects and CLL patients appeared to consist of a morphologically uniform population of small lymphocytes. Cells from the CLL patients in this study were generally of the same size or smaller than the normal lymphocytes.

Following incubation at 37°C for 3 hr, lymphocytes observed in the nitrocellulose filter matrix appeared to exhibit the distribution characteristic of a "random walk" process as described by Zigmond and Hirsch in their study of polymorphonuclear leukocytes,12 i.e.: a cross-sectional examination of the filters at each time point generally demonstrated a uniform distribution of cells, consisting of a leading edge of a smaller number of cells with progressively increasing numbers of cells closer to the surface of the filter (Fig. 1). In Zigmond and Hirsch's studies,12 quantification of the migration distance of the leading edge of such a distribution was a good indicator of the random motility of the entire population. At higher power observation of our filters, the cells were clearly seen to be mononuclear, but no consistent morphological differences could be demonstrated between cells closer to the leading edge and those closer to the surface of the filter in either the normal or CLL lymphocyte studies.

Motility of Purified Lymphocytes

As depicted in Fig. 2, when chambers were incubated for 3 hr at 37°C, lymphocytes from 20 normal subjects had a mean migration distance of 49.7 ± 2.7 μm into the filter. On the other hand, lymphocytes from 25 CLL patients had a mean migration of 32.5 ± 3.6 μm (p < 0.0005). (Migration distance was determined as described in Materials and Methods.) Incubation in the chambers for 18 hr at 37°C produced an additional, though not linear, increment in migration with the same highly significant difference in motility maintained
(Fig. 2). The nonlinearity of the increase in migration during the 18-hr incubation was a reproducible feature of the system. It did not appear to be due to cell death, since cells appeared intact morphologically in the filters and by trypan blue exclusion. However, the basal media used in these experiments may not have been sufficient to support the metabolic requirements for motility over such long time periods. Accordingly, only the 3-hr incubations were used for critical comparisons and to assess the effects of chemical agents.
In order to demonstrate that the migration into the filters was an active process, migration values were obtained from normal and CLL lymphocytes after incubation in the chambers for 3 hr at 4°C. Under these conditions, the normal mean migration was 8.8 ± 1.2 μm, while the CLL lymphocytes' mean migration was 7.7 ± 0.8 μm. At 18 hr, the mean value for normal subjects' lymphocytes was 11.6 ± 2.0 μm, while the CLL mean was 9.0 ± 1.1 μm. Thus there was a negligible difference between the 3-hr and 18-hr value and only a small degree of migration into the filter at this temperature.

Since the mean age of the normal subjects was 42 yr and that of the CLL patients 58 yr, it was of interest to determine whether the differences in migration could be accounted for by differences in age alone. In Fig. 3, individual subject values are depicted for the 3-hr and 18-hr incubations at 37°C. Normal subjects under the age of 30 are shown separately from those greater than age 40. It can be seen that there was no significant difference between these two age groups, thus discounting the possibility that the depressed locomotion found in the CLL patients' lymphocytes was solely age dependent. Figure 3 also demonstrates that although there was some overlap between the CLL and normal groups, almost one-half of the CLL patients fell more than two standard deviations outside the normal mean. Though most of the patients with CLL had lymphocytes that migrated well below the mean normal value, a small group of patients (four) had motility above the normal mean (Fig. 3). Table 1 shows the peripheral blood, clinical, and lymphocyte surface features of the CLL patients listed in decreasing order of cell motility. It can be seen that the four patients mentioned above were all in good clinical control with essentially normal blood counts and surface marker values approaching normal. This rapid motility may have reflected the migration of a normal population of lymphocytes at the leading edge of the total cell population. The patients with the slowest motility, on the other hand, tended to have much more abnormal blood counts and much higher percentages of surface Ig-containing cells, presumably indicating a more uniform population of CLL cells. Thus the magnitude of the motility defect is emphasized when one considers the extremely slow migration of the cells from patients with the most active CLL. As noted, there are both treated and untreated patients with all degrees of motility.

Fig. 3. Motility of individual normal and CLL subjects. Each circle represents an individual subject.
Table 1. Peripheral Blood, Lymphocyte Surface, and Clinical Characteristics of CLL Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>3-hr Motility (µm)</th>
<th>Peripheral Blood</th>
<th>Surface Markers</th>
<th>Clinical</th>
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<tr>
<td></td>
<td></td>
<td>WBC/cu mm</td>
<td>Lymphocytes (%)</td>
<td>Ig (%)</td>
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<td>75.5</td>
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The patients are listed in descending order of motility. The normal motility values ranged from 23 to 80 µm (Fig. 3). White blood counts and differentials were performed in a routine manner. The percentage of cells that contained surface membrane immunoglobulin by fluorescent labeling, and the percentage of cells forming rosettes with SRBC were determined as described in Materials and Methods. The normal range for per cent Ig containing cells is 5%–30% and for sheep rosetting cells 40%–70%. Cb, chlorambucil; P, prednisone; Cy, cyclophosphamide.
Table 2. Motility of Purified Normal Lymphocyte Subpopulations

<table>
<thead>
<tr>
<th>Cells</th>
<th>Depth of Migration (µm) 3 hr, 37°C</th>
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<tbody>
<tr>
<td>Total lymphocyte population</td>
<td>35.0 ± 2.6</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>47.3 ± 3.6</td>
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<tr>
<td>T lymphocytes</td>
<td>29.9 ± 2.7</td>
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Lymphocytes were purified from eight normal subjects as described in Materials and Methods, and B and T populations prepared by gradient centrifugation and rosetted lymphocytes as described.

Lymphocyte Subpopulation Locomotion

Because of the high proportion of lymphocytes containing surface Ig in the peripheral blood of patients with CLL, the cells have been considered by many as derivatives of the “bone marrow” derived or B-cell population. In order to examine the possibility that the decreased locomotion of CLL lymphocytes might represent a slower, inherent motility of B cells compared to that of T cells, partially purified (greater than 90%) populations of normal T and B cells were prepared. For eight normal individuals, the depth of migration achieved in 3 hr (37°C) was determined for the total lymphocyte population as well as the separated B- and T-cell populations. The results shown in Table 2 demonstrate that the B-cell preparation was actually more motile than either the T-cell preparation or the total lymphocyte preparation, while the T lymphocytes were slightly less motile than the total lymphocyte population. To determine whether the rosetting procedure for separating the T and B cells had altered the cells so that their migration characteristics were changed, the enriched T-cell population and B-cell populations from two normal subjects were remixed at a ratio of 3:1, respectively, thus simulating the approximate ratios in normal peripheral blood. When these reconstituted lymphocyte populations were studied after 3 hr of migration at 37°C, the mean migration value was 33.6 µm for these two subjects, and was not significantly different from that of the original total population (35.0 ± 2.6 µm) but lower than that of the B cells alone. The values for the total lymphocyte population migration determined in this set of studies could not be directly compared with the mean migration values of the larger group of normal subjects described above because of the differences in preparative technique and incubation medium described in Materials and Methods.

Effects of Chemical Agents on Motility of Normal and CLL Cells

Further confirmation that lymphocyte locomotion as measured in the modified Boyden chamber assay was an active energy requiring process (as suggested by the failure of migration at 4°C) was obtained by studying the locomotion of cells incubated with 10^{-2}-M sodium azide. In Fig. 4, the results are shown for purified lymphocyte preparations from five normal subjects and one patient with CLL. Equal portions of each subject’s cells were incubated in medium containing sodium azide or Gey’s medium (control). The results are expressed as the percentage of control migration achieved by cells migrating in medium containing sodium azide. Normal lymphocytes so treated migrated a mean of 15% of the control migration ($p < 0.001$). After incubation in the medium containing sodium azide for periods of time equivalent to that of the
migration incubation, 95% of the lymphocytes were viable by trypan blue exclusion.

In order to characterize further the migration process, two drugs reported to affect cell locomotion, colchicine, and cytochalasin B, were studied in a manner similar to that for sodium azide to determine their effects on migration into the filter. Lymphocytes from four normal subjects and four CLL patients were incubated in the presence and absence of cytochalasin B (10 μg/ml). Normal control migration was 58 ± 5.7 μm, while incubation with cytochalasin B decreased it to 5.7 ± 3.0 μm or 8.7% of the control (p < 0.0005). For the CLL patients thus studied, control migration was 35.7 ± 6.4 μm, while it was 8.8 ± 1.9 μm for the cytochalasin B incubated cells, or 15.8% of control (p < 0.02). Control migration for cells from eight normal subjects in the absence of colchicine was 58.0 ± 5.7 μm, while incubation in the presence of 10^{-3} M colchicine increased this to 84.5 ± 6.9 μm over a 3-hr period or 148% of control (p < 0.0005). For the seven CLL patients thus studied, the control mean migration was 35.7 ± 6.4 μm. Incubation in the presence of 10^{-3} M colchicine produced migration of 61.9 ± 7.5 μm for an increase of 189% of control (p < 0.0005). Similar incubations were carried out in the presence of vincristine and vinblastine, demonstrating that migration into the filter was increased to essentially the same degree as demonstrated for colchicine (Fig. 4). Once again, for each of these agents incubations of cells in the presence of the medium containing the chemical agent, for periods equivalent to that employed for the study of locomotion, yielded cells that were 95% viable by trypan blue exclusion.

Since this method quantifies motility by an assessment of the depth of penetration of the leading group of cells, changes induced in the faster moving cells might be most obvious and an effect, or lack of effect, on a slower moving population obscured. However, in the inhibitor studies reported here the depth of penetration was reduced to almost nil, so that the entire cell population must have been affected. In the experiments demonstrating an increase in motility, the migration appeared random in the filters, and no separation of a group of cells was noted. However, stimulation of only the normally slower moving cells could not be excluded.
DISCUSSION

The Boyden chamber system or a modification thereof has been used to measure chemotaxis of polymorphonuclear leukocytes and monocytes and, more recently, as a measure of random locomotion of the same cell types. Zigmond and Hirsch have demonstrated that these cells exhibit a "random walk" type of behavior, and that the distance moved by the leading edge of the cell population as designated by the two front-moving cells was a true indicator of the movement of the cell population at large. Keller et al. have indicated that active passage of lymphocytes could be demonstrated in such a system and that it might be useful in the assessment of lymphocyte motility. Schreiner and Unanue have demonstrated, in addition, that the random-walk analysis of the penetration of mouse lymphocytes into such filters could be useful in the determination of anti-Ig effects on lymphocyte motility. We have applied this technique to the measurement of random locomotion of human lymphocytes. Previous assays of lymphocyte motility have depended upon studies involving translational movement along a glass slide surface after prolonged periods in tissue cultures, or upon the morphology exhibited by such cells after incubation on glass slides. In the latter type of assay the presence of uropod structure has been taken to indicate motile cells, although these features do not always correlate. The micropore filter assay enables one to study this function under more physiologic conditions of short-term incubation. In this manner we have been able to obtain reproducible measurements of lymphocyte motility and alterations thereof induced by disease, drugs, or decreased temperature.

Utilizing the slide incubation method, Schrek demonstrated decreased motility of CLL cells after three days in culture. However, the assay was unable to detect any motility of normal or CLL cells during the first 24 hr, thus introducing the possibility that the cells may have undergone alterations during culture. Furthermore, due to the prolonged periods in tissue culture and the requirement for laborious plots of linear measurements of individual cells, only a small number of cells could be studied. Utilizing the filter migration assay, we have demonstrated that chronic lymphocytic leukemia lymphocytes are significantly less motile than normal cells during short-term incubation. This finding was not attributable to age differences among the subjects, since lymphocytes from younger and older normal control donors had significantly greater motility than CLL cells. The low migration capacity of CLL cells was not likely due to size difference, since the cells were generally smaller than normal cells, an observation made by others as well, and on a passive basis might be expected to "fall" through the filter even more rapidly. Moreover, the marked decrease in migration produced by sodium azide and decreased temperature indicated the active nature of the process being measured in both instances.

Normal lymphocytes with surface properties assumed to define thymus-derived (T) or bone-marrow-derived (B) cells differ in a number of physiologic characteristics such as adherence to glass surfaces and nylon fibers and electrophoretic mobility. Most CLL cells studied have had surface properties similar to those presumed to be of B-cell origin, and thus motility differences could conceivably result simply from enrichment of the percentage of such cells in the CLL patients. However, in our study, purified normal B cells
proved to be more motile than T cells and combined normal cell populations. Thus, by comparison CLL cell motility was markedly reduced with respect to normal B-cell motility, supporting the concept that the CLL cell is intrinsically abnormal with respect to motility. Moreover, since the separated normal B cells migrated faster than the T cells, they would tend to be at the advanced edge of the total lymphocyte population for comparison with the CLL cell motility. These results must be viewed with some caution, since the separated normal populations were not absolutely pure. However, the concept of an intrinsic abnormality was further supported by the finding that among CLL patients, cells from those individuals with presumably purer populations of abnormal cells had the slowest motility, while those from patients with the largest portion of remaining normal cells had the higher motility, thus emphasizing the slow motility of the CLL population per se.

Microfilaments have been noted in proximity to the plasma membrane of lymphocytes,\textsuperscript{24,25} and have been implicated in cell locomotion of other cell types\textsuperscript{26} because of the dramatic inhibition of cell locomotion caused by cytochalasin B, which inhibits microfilament activity.\textsuperscript{15,25} We have noted the same effect on human lymphocytes (both normal and CLL), suggesting a role for microfilaments in the locomotion of these cells as well. Though cytochalasin B has been noted to have other effects such as decreased glucose transport and glycoprotein synthesis,\textsuperscript{15,25,27,29} such effects have been felt not to be limiting over the short time period (3 hr) used in this study.\textsuperscript{25,29} Microtubules appear to be of great importance in the maintenance of a cytoskeleton,\textsuperscript{30} intracellular organelle movement,\textsuperscript{3} and topographic stability of the membrane.\textsuperscript{32} However, studies of the effects of inhibitors of microtubular activities, such as colchicine and the vinca alkaloids, upon cell motility have been contradictory, and, while some have shown a decrease in sustained directional movement,\textsuperscript{16} others have shown no inhibition.\textsuperscript{33,35} Human lymphocytes have not been studied in this regard. In our study, colchicine, vincristine, and vinblastine all produced a significant increase in human lymphocyte (CLL and normal) movement into the filter. However, rather than causing an actual increase in the forces of locomotion, microtubular inhibitors may reduce the resistance offered by the intact cytoskeleton and allow the cell to navigate the convoluted pores more easily because of increased distensibility. The evidence would certainly indicate, however, that microtubules are not required to sustain locomotion of human lymphocytes.

A number of studies have now demonstrated the independence of cell motility defect of human CLL lymphocytes.

![Fig. 5. Hypothetical mechanism for the capping and motility defect of human CLL lymphocytes.](image)
ity and capping in animal cells. In our studies of human lymphocytes

cytochalasin B did not inhibit capping, though it did effectively inhibit motility

in the same dose range. Colchicine, while inhibiting normal human lymphocyte

Ig capping, did not inhibit motility. The data on cytochalasin B and colchicine
effects presented here and previously suggest that microfilament integrity is

essential for human lymphocyte motility, but not for surface Ig redistribution,

while microtubular components of the system may be of greater importance in

human lymphocyte surface Ig redistribution than in locomotion.

Since the phenomena of membrane redistribution and motility appear to be

independent in normal human lymphocytes, the anomalous surface immuno-
globulin Ig capping of CLL cells previously reported would not appear to be
cauised by the decreased motility of these cells demonstrated in this study. The
two abnormalities, however, could still be caused by a single defect. If the
microfilament–microtubular structures in human lymphocytes exist as a related
system, as has been suggested for other cells, a defect at a microtubular–

microfilament junction as depicted in Fig. 5 could result in the abnormalities
described, while defects or inhibition of each structure individually would re-
sult in isolated disorders. Further resolution of this question will depend upon
better morphological and physiologic definition of the relationships of micro-
tubules and microfilament structures in normal and CLL lymphocytes.

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