Time-Dependent Alterations in the Deformability of Human Neutrophils in Response to Chemotactic Activation

By Robert S. Frank

Transit times of individual human neutrophils through single capillary-sized pores were measured to determine the time-dependent changes in the mechanical behavior of the cells during activation by the chemotactic agent formyl-methionyl-leucyl-phenylalanine (FMLP) and in response to cytochalasin B (CTB) and colchicine. FMLP elicited a two-phase response consisting of a rapid increase in cell stiffness, which peaked between 2 and 3 minutes, followed by a partial recovery of deformability to a level significantly above that of control after 5 minutes. The mechanical changes closely followed changes in F-actin content, although the peak in cell stiffness appeared to lag the F-actin response. Treatment with 3 μmol/L CTB produced a transient decrease in cell rigidity followed by a return to control level in 10 minutes, whereas treatment with 30 μmol/L CTB resulted in a sustained decrease in cell transit times to a level 60% of control. Addition of 3 μmol/L CTB to cells prestimulated with FMLP produced a rapid (1 to 2 minutes) cessation of changes in cellular deformability produced by the FMLP. Colchicine treatment did not decrease cell rigidity, but produced a delayed increase in F-actin content accompanied by increased stiffness of the cells. These results implicate actin as the major determinant of the mechanical behavior of the neutrophil, as measured by whole cell deformability tests. The significant changes in cell deformability that occur in response to fractional changes in F-actin content suggest that changes in the structure of the actin network occur during these processes.

© 1990 by The American Society of Hematology.

The mechanical responses of polymorphonuclear neutrophils and other white cell types has become an area of intense study in recent years. These cells exhibit a variety of self-directed, active behaviors that require rapid rearrangement of their molecular components. A sizeable body of evidence points to contractile actin and the actin-myosin complex as the major proteins involved in locomotion, phagocytosis and exocytosis. Actin, one of the most abundant proteins in the neutrophil, exists in two conformations; the monomeric globular form (G-actin), and the filamentous form (F-actin) that polymerizes into 7-μm-diameter fibers called microfilaments. The microfilament network is located mainly in the periphery, or cortex, of the cells. Present in the cytoplasm of these cells are a number of proteins that interact with the actin monomers or filaments and act in concert to regulate the structure, and thus the mechanical behavior, of the actin network. Profilin bonds to actin monomers and acts to prevent the formation of new actin filaments and to stabilize the length of existing filaments. Gelsolin can cap the rapid growth end of the actin filament and sever filaments. Actin-binding protein crosslinks actin filaments orthogonally and can thus create isotropic actin networks. Depending on the concentration of actin fibers, the fiber length, and the amount of crosslinking, the microfilament network exhibits either liquid-like or solid-like behavior. This sol-to-gel transition results in major changes in the mechanical behavior of the actin network. Although in vitro studies have shown that actin monomers and uncrosslinked polymers exhibit viscoelastic behavior, a significant increase in rigidity and viscosity occurs during gelation of the network. The elastic behavior of monomer solutions has been attributed to weak intermolecular bonding between actin molecules; the elastic properties of uncrosslinked polymers have been attributed either to these same weak intermolecular bonds or to the entanglement of the actin fibers. In the gel state, the actin network is rigidly crosslinked by a secondary protein, such as actin-binding protein.

Significant progress has been made in measuring and modeling the mechanical behavior of the neutrophil itself. In its passive state, the neutrophil exhibits both elastic energy storage and liquid-like behavior. Current models describe the cell as a viscous or viscoelastic liquid surrounded by a cortical layer that is under tension. This tension is of a small magnitude but is present in the undeformed, spherical cell and is thought to act as the driving force for the recovery of geometrically deformed cells back to the spherical shape. Morphologically, this tension is attributed to the actin-rich layer in the cortex of the cell. The consistency of the cortex in the passive state is not known. The existence of a persistent tension would argue for some degree of gelation. However, the cortex will flow on prolonged aspiration, and its tension is thought not to increase during deformation.

During activation of the neutrophil, rapid and significant changes occur in the amount of polymerized actin, the structure of the microfilament network, and the mechanical behavior of the cells. Experimental protocols often use chemotactic peptides such as formyl-methionyl-leucyl-phenylalanine (FMLP) to induce cell activation. In the initial phase of the chemotactic response there is a rapid conversion of G-actin to F-actin. The maximal response, such as that induced by FMLP in concentrations of 1 nmol/L or greater, produces an ~100% increase in F-actin content within 2 minutes. Fluorescent micrographs have shown that initially the additional F-actin adds broadly to the submembrane cortex. Morphologically, during this stage the neutrophils remain spherical in shape but form numerous ripples and exhibit no directed motion. In the next several minutes of this response, total F-actin content decreases from this transient peak to a level greater than that of the...
unstimulated cells. During this phase, the F-actin content of the cortex decreases, and pseudopods, which are rich in F-actin, form. Pseudopods have been shown to consist of a network of orthogonally crosslinked microfilaments, and micropipette studies have shown them to be quite rigid. The locomotive response also requires a contractile process to retract the pseudopod. Contraction has been postulated to occur within the pseudopod and is thought to involve the actin-myosin complex.

Several investigators have measured the mechanical behavior of FMLP-activated neutrophils through a variety of techniques. These studies typically revealed a dose-dependent increase in the rigidity of FMLP stimulated cells. Worthen et al used their “cell poker” to measure cell stiffness. They noted as much as a seven-fold increase in the average stiffness of cells in response to 1 μmol/L FMLP. They also noted a heterogenous response to stimulation, with a significant portion of the population not responding. Kawaoka et al measured the minimum pressure necessary for aspiration of the neutrophil into micropipettes during the time period of 15 to 75 minutes after stimulation with FMLP. For FMLP concentrations of 5 mmol/L and below, there was a decrease in the pressure required for whole cell aspiration during this period; for concentrations of 10 nmol/L and above, there was greater cell stiffness. To date, the time course of the mechanical response to FMLP has not been clearly detailed in any existing reports.

Cytochalasin B (CTB) and the other cytochalasins known to interact with the actin network have been used as an intervention for studying both actin networks in vitro and actin regulation in the neutrophil. The cytochalasins are thought to bind to the rapid growth end of the actin polymer in a manner similar to that of gelsolin. Its action, however, is not calcium dependent. This binding results in a reduction of filament length and has been shown in vitro to be capable of causing the solation of an actin gel. In neutrophils, CTB does not affect total F-actin content but causes a reduction in Triton-insoluble cytoskeletal-associated actin. Pretreatment of cells with CTB will inhibit the F-actin increase normally produced by FMLP. Worthen et al found preincubation of neutrophils with cytochalasin D prevented increases in both cell stiffness and retention of cells in 5-micron-diameter pores normally produced by FMLP stimulation.

Microtubules are a second component of the neutrophil that may be mechanically significant. Colchicine is an agent that disrupts the microtubules and can be used to evaluate their contribution to the mechanical behavior of the cells. A previous study by Chien et al showed significant reductions in both cellular viscosity and elasticity during the initial, limited aspiration of projections of the cell into small diameter micropipettes.

The objective of this study is to detail the specific time course of the mechanical changes of neutrophils in response to stimulation with FMLP and exposure to CTB and colchicine. A single pore transit time analyzer is used to measure the passage time of individual neutrophils through a 5-micron-diameter pore. The individual transit time serves as the measure of the mechanical state of each cell at the time of evaluation. Because the measurement time for each cell is short (on the order of 1 second) relative to the minute time scale for chemotactic activation, it is possible to follow the changes in the cell population over this time period. Evaluation of the time course of mechanical changes will be correlated with changes in actin content and structure to further elucidate the specific changes occurring during these complex processes.

**MATERIALS AND METHODS**

Approximately 10 mL of whole blood, obtained by venepuncture from healthy adult volunteers, was drawn into vacutainers containing heparin as the anticoagulant. Granulocytes were separated from the whole blood using a Ficoll-Hypaque density separation medium (Mono-Poly Resolving Medium, Flow Laboratories, McClean, VA). Approximately 3.5 mL of the heparinized blood was layered on top of 3 mL of the separation medium in each of two 13 x 100 mL test tubes. The tubes were spun at 300g for 30 minutes in a swinging bucket rotor at room temperature. The lower white cell band, which contained the granulocytes, was removed by aspiration with a needle and syringe. Cells were resuspended in an isotonic phosphate buffered saline solution (PBS) containing 121 mmol/L NaCl, 25.2 mmol/L Na2HPO4, 4.7 mmol/L KH2PO4, and 5.6 mmol/L glucose. Penicillin (100 U/mL) and streptomycin (100 μg/mL) were added to the buffer to retard bacteria growth. Bovine serum albumin was added at a concentration of 1.0 g/L. Solution osmolarity was between 290 and 300 mOsm, as measured by a vapor-pressure osmometer (Wescor Corporation, Logan, UT); pH was 7.40 ± 0.05. Before use all solutions were filtered through a 0.2 μm filter (Gelman Sciences, Ann Arbor, MI) to remove bacteria and debris. Cells were washed by centrifugation and resuspended in fresh buffer two times. After the final wash, the cells collected from the two original separation tubes were resuspended in 5 mL of fresh buffer at a concentration of approximately 10⁶ cells/mL. The cells were a mixture of approximately 75% white cells and 25% red cells. Of the white-cell fraction, approximately 95% to 97% were neutrophils, as determined with Wright’s stain. The resistive pulse measurement of mechanical behavior easily distinguishes between white cells and red cells, so no further purification of the white cells was necessary.

Deformability of the white cells was assessed by measuring the time required for the cells to completely travel through a single, capillary-sized pore in a thin polycarbonate membrane (Nuclepore Corp, Pleasanton, CA). Pores used in this study had diameters of 5.0 (±0.2) microns and lengths of 10.5 (±0.5) microns. Cells were driven through the pore by a pressure of 1 kPa (10 cm H2O) imposed across the polycarbonate membrane. A DC potential of .2 to .3 V was imposed across the polycarbonate membrane, and the change in electrical resistance produced across the membrane as the white cell entered and transited the pore was measured and recorded digitally for subsequent analysis. The pore-containing membrane was mounted on a light microscope, and the transit of the cells was visually monitored. The pore was ultrasonically cleaned when it was observed, either visually or from the resistive pulses, that cells were adhering to the pore or that the pore was obstructed. Resistive pulses indicating the cell had adhered to the pore were discarded. Details of this apparatus and its use with white cells has been described previously. For studies involving time-dependent alterations in mechanical behavior, the time elapsed between the specified treatment of the cell and its entrance to the pore was recorded along with the time required for the cell to transit the pore. All measurements were performed at room temperature and were completed within 6 hours of venepuncture.

A stock solution of 200 μmol formyl-methionyl-leucyl-phenylalanine (FMLP) in dimethylsulfoxide (DMSO) was prepared. Before
use, the stock was diluted in PBS to concentrations of 11.1, 1.1, .11, and .011 nmol/L. Cytochalasin B was dissolved in DMSO at a concentration of 10 mmol/L and diluted before use in PBS to concentrations of 3.3 and 33.3 μmol/L. The highest concentration of DMSO to which the cells were exposed was .4% vol/vol. Transit time of cells exposed to this concentration of DMSO was measured as a control. A stock solution of 1 mmol/L colchicine in PBS was also prepared.

For experiments testing the response of neutrophils to FMLP, 1 mL of the cell suspension was mixed with .9 mL of the appropriate FMLP solution. This resulted in the final FMLP concentration as reported. Cells were quickly injected into the measurement chamber, and the transit times of the cells were measured as a function of time after mixing with the FMLP. The first cell typically entered the pore within 15 seconds after exposure to FMLP. Measurements were made for a period of 10 minutes. For statistical purposes, the transit times of all cells that entered the pore in each 1-minute interval were grouped together. The experimental protocol was repeated until the transit times of a minimum of 50 cells were recorded for each 1-minute interval. This group of measurements constitutes a single experiment. Transit times of unstimulated cells were measured as a control for each experiment, and all results were normalized by the control transit time for that experiment. Experiments measuring the response of cells to CTB were performed in the same manner as described above, except that cell transit times were grouped into 2-minute intervals and measurement were made for a period of 20 minutes. Transit times of these cells were also measured at 120 minutes poststimulation. The response of cells to 10 μmol/L and 100 μmol/L colchicine was measured over a 20-minute interval, with data presented in 5-minute groups. Cells pretreated with CTB and then stimulated with FMLP were tested between 20 and 120 minutes posttreatment with CTB. Again, measurements were grouped into 2-minute intervals. The final protocol involved exposure of cells to 3 μmol/L CBT 1 minute after stimulation with either .1 or 1 nmol/L FMLP. Cells were stimulated with FMLP in the usual manner, but were not initially injected into the measurement chamber. After 1 minute, the 1 mL of stimulated cells was mixed with .1 mL of 33 μmol/L CBT resulting in a final CBT concentration of 3 μmol/L.

Cells were then injected into the measurement chamber and transit times were recorded for 5 minutes. A separate set of FMLP experiments was performed as controls.

Measurements of changes in the F-actin content of the neutrophils by flow cytometry were performed simultaneously during selected experiments of .1 nmol/L FMLP and colchicine treatment. Stimulated cells were fixed in 3.2% paraformaldehyde at the appropriate time interval after addition of either FMLP or colchicine during the period in which resistive pulse measurements were being made. Cells were subsequently stained with NBD-phallacidin, and fluorescence intensity histograms were determined with an EPICS V flow cytometer (Coulter Electronics, Hialeah, FL) interfaced to a Coulter MDADS data acquisition system. Fluorescence staining and measurement were performed by the laboratory of C.H. Packman, and details of their procedure are published.

**RESULTS**

Initial experiments evaluated changes in neutrophil deformability in response to stimulation with the chemotactic compound FMLP. Figure 1 details the histograms of the transit times of unstimulated neutrophils and the transit times of cells during specific time intervals after stimulation with .1 nmol/L FMLP. Data shown is from a single experiment. The histogram of the control (unstimulated) group in Fig 1A is typical of the skewed distribution seen in the transit times of the unactivated neutrophil population.

The majority of cells are closely grouped around the median transit time (~4 seconds in this case), and a small number of cells display significantly longer transit times. All cells with transit times of 4 seconds or longer, and cells that do not completely pass through the pore, are shown in the rightmost interval in all the histograms in this figure. During the experimental measurements it was observed that some cells would not completely travel through the pore. To facilitate data collection and prevent adhesion of cells to the pore, a maximum residence time of approximately 10 times the median transit time was established. Cells not completing their transit through the pore in this interval were removed from the pore and the event was recorded as a "non-passage." Figures 1B through 1F show the transit times of cells that entered the pore during the first minute after stimulation with FMLP, during the second minute, the third minute, between 5.5 and 6.5 minutes, and between 8.5 and 9.5 minutes, respectively. These results show that a significant fraction of the cells (~40%) became much stiffer within the first 2 minutes of stimulation. This response was followed by a relaxation of cell stiffness over the next several minutes. A significant fraction of the cells (~30%) did not exhibit a detectable response to the FMLP.

Time-dependent response of neutrophils to stimulation with FMLP is shown in Fig 2. Results from each experiment are normalized with respect to the transit times of unstimulated cells for that experiment (.38 to .46 seconds). Results from treatment with .1 nmol/L FMLP (three experiments), .01 nmol/L FMLP (two experiment) and .4% vol/vol DMSO (two experiments) are plotted. Points represent the average of the median transit time for each experiment, and error
MECHANICAL RESPONSE OF NEUTROPHIL ACTIVATION

Fig 2. Time-dependent transit times of cells exposed to .1 nmol/L FMLP (3 experiments), .01 nmol/L FMLP (2 experiments) and .4% vol/vol DMSO (2 experiments). Data shown are the averages of the median transit times for each experiment and error bars represent standard deviations of the medians. All data are normalized by transit times of untreated cells.

Fig 3. Time-dependent % increases in neutrophil transit time (Δ, right vertical scale) and F-actin content (○, left vertical scale) after stimulation with .1 nmol/L FMLP (2 experiments). All data are normalized by values for unstimulated cells.

were tested. Figure 4 shows the results of treatment of the cells with 3 μmol/L CTB (five experiments) and 30 μmol/L CTB (four experiments). Again, results are normalized by the median transit time of untreated cells; points represent the average of the medians, and error bars represent the standard deviation of the medians. Cells treated with 3 μmol/L CTB showed an initial decrease in transit time of ~30% followed by a gradual return to the baseline level. Cells exposed to 30 μmol/L CTB showed a rapid decrease in transit time of ~50% followed by a slow recovery to a level ~60% of control. The response that each group exhibited at 20 minutes persisted when tested at 120 minutes poststimulation (data not shown). Figure 5 details the response of cells pretreated with CTB and subsequently exposed to 1 nmol/L FMLP. Data shown are from four experiments at each level of CTB pretreatment. Each group showed the typical response to FMLP activation, but the increase in transit time caused by the FMLP was greatly attenuated relative to the response of cells not exposed to CTB. Cells pretreated with 3 μmol/L CTB showed a significant increase in transit time

The role of actin in the mechanical changes in response to .1 nmol/L FMLP stimulation was evaluated by simultaneously measuring deformability changes and changes in F-actin content. Results of two experiments are plotted in Fig 3. The F-actin response closely follows the mechanical changes, although increases in transit times of up to 4-fold occur with only a 35% increase in F-actin content. The time response of the initial stiffening appears to lag the F-actin increase; however, it should be noted that the F-actin measurements are "instantaneous" as cells were fixed at the time point indicated, whereas deformability measurements were made over a 1-minute interval centered at the time point plotted. Histograms of fluorescence intensity (and thus F-actin content) showed some skewing upon activation, with a small shoulder developing at right (low) side. However, the bimodal distributions seen in the transit time histograms are not seen in these histograms.

Time-dependent changes in the deformability of neutrophils in response to concentrations of cytochalasin B (CTB)
Fig 5. Time-dependent transit times of cells pretreated with CTB then stimulated with 1 nmol/L FMLP. Results shown are from 4 experiments at each pretreatment level. Data shown are the averages of the median transit times from each experiment and error bars represent standard deviations of the medians. All results are normalized by transit times of untreated cells.

after 2 minutes, followed by a recovery to a level not significantly different from the pretreatment level. Cells pretreated with 30 μmol/L CTB produced a very slight increase in stiffness in response to the 1 nmol/L FMLP followed by a reproducible decrease in transit time to a level consistently below the established CTB baseline.

Treatment of cells with CTB after the chemotactic response was initiated served as a final method to investigate the role of actin in the chemotactic response of the neutrophil. Figure 6 shows the response of cells stimulated with 1 nmol/L FMLP then treated with 3 μmol/L CTB 1 minute after the FMLP stimulation. The data shown are from three experiments. Addition of CTB 1 minute after stimulation with 1 nmol/L FMLP resulted in a rapid blocking of the FMLP response and the recovery within 1 minute of the cell’s deformability to the level of the unstimulated cells. The morphology of the cells also recovered to the smooth spherical shape after CTB treatment, although restoration of the cell’s deformability occurred faster than did its shape recovery. Figure 7 shows the behavior of cells treated with 3 μmol/L CTB after stimulation with 1 nmol/L FMLP (three experiments) as well as the results previously shown in Fig 5 of cells stimulated with .1 nmol/L FMLP. Cells activated with 1 nmol/L FMLP returned to the behavior of unstimulated cells within 2 minutes after treatment with 3 μmol/L CTB.

Finally, the role of microtubules in the mechanical behavior of the neutrophil was evaluated by measuring the time-dependent response of these cells to colchicine. Doses of 10 and 100 μmol/L were chosen to compare these results to those of Chien et al. There was no significant decrease in cell transit times in response to either dose of colchicine as seen in Fig 8A (four experiments). Additionally, cell stiffening and the formation of protopodia was consistently noted at the longer time points in response to the 100 μmol/L dose, and occurred once with the 10 μmol/L dose. Measurement of F-actin content during one of these experiments showed that this stiffening was again accompanied by an increase in F-actin (Fig 8B). Again the magnitude of the F-actin increase was a maximum of 20% whereas transit times increased by a maximum of over ten-fold.

DISCUSSION

Resistive pulse measurements of the transit times of white cells through a single-pore membrane serve as a unique method to study time-dependent changes in the mechanical response of the white cell population. The ability to make rapid measurements of a single index (ie, transit time) allows the collection of the large amount of data necessary for statistical analysis of a cell population that exhibits nonhomogeneous mechanical behavior. Furthermore, the visual observation of each cell’s transit and the review of each resistive pulse allows discrimination between extended transit times due to changes in the behavior of the cell and artifactual changes that may occur because of adhesion of the cell to the membrane or plugging of the pore.

These results show the remarkable ability of the neutrophil to undergo rapid and substantial changes in its mechanical
MECHANICAL RESPONSE OF NEUTROPHIL ACTIVATION

state and implicate microfilaments as the primary structure responsible for these changes. Stimulation with FMLP produces a rapid rigidification of the cells within 1 to 2 minutes. At low levels of FMLP exposure (1 nmol/L), ~35% of the cells exhibit major rigidification. In our studies, F-actin levels increase a maximum of ~35% during this response. This result is similar to the ~50% increase in F-actin seen by others at this FMLP level. At higher levels of FMLP exposure a larger percentage of cells is affected and the degree of rigidification is greater. During this initial period the F-actin is distributed throughout the cortex. Cells remain spherical but exhibit greater surface roughness. During the next several minutes, overall cell deformability increases, total F-actin decreases, pseudopods begin to form, and the F-actin is concentrated in the region of the pseudopods.

Of considerable interest in these results is the magnitude of the mechanical response vis-a-vis the magnitude of the F-actin response, and the observations of reacting and nonreacting populations in this response. Although white cells are much more difficult to deform than red cells, the passive cells are primarily liquid-like in behavior. Weak elastic and viscoelastic components are also present in the cells.) The time required for a cell to enter into a capillary, be aspirated into a micropipette, or transit a capillary pore (as measured in these studies) is dominated by and proportional to the cellular viscosity. During activation, some cells exhibit increases in transit times of 10-fold and greater, yet some cells will not transit the pore at all. This nonlinear response is most probably due not to an increase in cellular viscosity, but to an elastic rigidification of the cell. This change, probably caused by a gelation of the actin network, results in an elastic rather than viscous material. Elastic behavior has been seen in gelated actin solutions as well as in stimulated neutrophils with the "cell poker" and in neutrophil pseudopods by micropipette techniques. Gelation can occur with just small changes in F-actin concentration; thus this phase change would allow large changes in cellular deformability in response to the fractional changes in F-actin content measured. The bimodal mechanical response is not inconsistent with the lack of a bimodal F-actin response, but probably is the result of this gelation's occurring in just a fraction of the cells. Worthen et al also observed a bimodal mechanical response in the neutrophil population. (They also reported that the decreased ability of FMLP-stimulated neutrophils to transit pores in polycarbonate membranes is not the result of increased adhesion of the stimulated cells to the membranes.)

Treatment of neutrophils with CTB also produced rapid and substantial changes in the mechanical behavior of the cells. Treatment of unstimulated neutrophils with 3 μmol/L CTB resulted in a transient decrease in the transit times of the cells followed by a return to the behavior of the unstimulated cells. This level of CTB exposure is reported to produce no significant change in either total F-actin content or the amount of cytoskeletal-associated actin. Exposure to 30 μmol/L CTB produced a large, persistent decrease in the cell transit time. This level of CTB has been seen to produce a 20% to 30% decrease in cytoskeletal actin. Measurements of the viscosity of neutrophils in response to CTB treatment, obtained from the deformation rate as a function of aspiration pressure, have shown that exposure to 30 μmol/L CTB produces a persistent decrease in cellular viscosity of ~55%. These results suggest that the cortex of passive neutrophils is largely in the solated state. Viscosity reduction may be accomplished by decreasing mean filament length. Pretreatment of neutrophils with CTB before FMLP stimulation substantially eliminates the deformability changes associated with the chemokinetic response. F-actin content does not increase, presumably preventing the microfilament network from rigidifying. Normal morphologic changes associated with the chemokinetic response are also not seen. It should be noted that in this protocol cells receive a longer exposure to CTB. Thus the inhibition of glucose transport by CTB may play a role in the diminished chemokinetic response.

The exposure of neutrophils to CTB 1 minute after initiation of the FMLP response produced a very abrupt interruption of the chemokinetic response and a rapid return of the cells to a mechanical state very near that of the unstimulated cells. The concentration of CTB used in these experiments, 3 μmol/L, is a level that changes neither the equilibrium level of cytoskeletal actin nor cellular deformability. It was also observed in these studies and in studies in which the chemokinetic response was interrupted after 5 minutes, that changes in cellular deformability occur before the cells revert to their unstimulated morphology. The structure of the cells appears to literally melt—an event probably associated with the solation of the crosslinked microfilament network. Residual elastic components in the cells then drive the recovery of cells that have been deformed.
by either passage through the micropore or by the formation of pseudopods back to a spherical shape. The magnitude of the cortical tension of CTB-treated cells is not known.

The response of cells to colchicine treatment revealed two important results. First, no significant decrease in transit time was seen, indicating that microtubules do not play a significant role in whole cell deformability (and bulk cellular viscosity) as measured in these experiments. Although this result is contrary to that of Chien et al,25 their procedure was a limited aspiration test that may measure membrane properties rather than bulk cellular viscosity. Second, the rigidification of the cells at longer times, which was paralleled by an increase in F-actin and the formation of pseudopods, shows that microtubules are not necessary for an activation response. (It should be noted that Chien et al also saw pseudopodia formation at long times [45 minutes] after high doses [150 amol/L] of colchicine treatment.)

In conclusion, time-dependent measurements of cell deformability serve as a valuable technique to examine the effect of molecular reorganization on the mechanical behavior of white cells. These studies clearly reveal the two-phase nature of the mechanical response of neutrophils to chemotactic stimulation. The strong correlation between total F-actin content and cell deformability implicate the actin microfilaments as the major structural elements involved in the activation of the neutrophil. The marked rigidification of the cells during the initial phase of the chemotactic response and the effect of CTB on these activated cells strongly suggest a gelation of the microfilament network occurs during activation. The effect of CTB on the passive neutrophils suggests the microfilament network in the passive cells is more liquid-like than gel-like. The mechanisms responsible for the persistent tension in the cortex of passive cells and those driving the recovery of CTB-treated cells is unclear. Future studies to address these questions are clearly necessary.

REFERENCES

Time-dependent alterations in the deformability of human neutrophils in response to chemotactic activation

RS Frank