Locomotion forces generated by a polymorphonuclear leukocyte

Shunichi Usami,* Shu-Li Wung,* Boguslaw A. Skierczynski,‡ Richard Skalak,‡ and Shu Chien‡
*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC; and ‡Institute for Biomedical Engineering and Department of Applied Mechanics and Engineering Sciences, University of California, San Diego, La Jolla, California 92093 USA

ABSTRACT There have been very few studies which have measured the physical forces generated by cells during active movements. A special micropipette system has been designed to make it possible to observe cell motion within the pipette and to apply a pressure to counter the chemotactic migration of the cell. This provides a direct measure of the locomotion force generated by the cell. The average velocity of forward motion is 0.33 μm/s in the absence of counter-pressure. The application of a positive counter-pressure (C-P) causes a decrease in the velocity of the forward motion of the cell. At 17 cm H_2O of C-P, the cell velocity drops to zero and even moves backward with a higher C-P. The results show that the decrement of velocity is linearly related to the magnitude of the C-P with a complete stoppage at a pressure of 17 cm H_2O which corresponds to a force of 0.003 dyn. The maximum work rate of the cell is $\sim 2.5 \times 10^{-8}$ erg/s.

INTRODUCTION

Cell motility has been the subject of many previous studies focused mainly on the kinematics of cell migrations, the biochemistry and motion of particular components (e.g., cytoskeletal proteins and plasma membrane), or adhesion mechanisms (Holifield et al., 1990). In contrast, there have been very few studies which provide experimental measurements of the physical forces generated by cells during active movements. In a recent review, Holifield et al. (1990) state that not only the magnitude of forces, but also the molecular mechanisms by which motile cells move their plasma membranes forward, remain largely unknown. The present experiments were performed to shed some light on the subject.

MATERIALS AND METHODS

Blood was drawn from healthy human volunteers into sterile disposable syringes with 10–20 U of sodium heparin per milliliter of blood. Blood was sedimented by gravity and leukocytes were obtained from the leukocyte-rich plasma. From these leukocytes, a polymorphonuclear leukocytes (PMNs) fraction was isolated using a density isolation technique. The PMNs were washed three times and resuspended in medium (Hank's buffer solution + 30 mM Hepes, pH 7.4) before their use in micropipette tests.

A special micropipette system has been designed which is able to induce a PMN to move into a micropipette with a tip of 5 μm without the application of negative pressure (Fig. 1). A small (#26) gage needle containing 10^{-6} M *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) in 1% gelatin is inserted into the rear side of the micropipette and kept at a distance of 3 cm from the micropipette tip. The convective current resulting from needle insertion causes fMLP to reach the micropipette tip and create a chemotactic source. In some cases, the micropipette was coated with fibronectin. Because the results were not distinguishable between experiments using fibronectin-coated and uncoated micropipettes, the data were combined in the presentation.

The tip of the micropipette was placed near a PMN and then the cell was allowed to remain there for a holding period. After 1–2 min, the PMN started to move into the micropipette and advance towards the fMLP source. This medium-filled micropipette was mounted on a hydraulic micromanipulator and its side port connected to a pressure generation system. The PMN suspension was loaded into a small chamber, located in a thermo-element temperature control device. This chamber was placed on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and the cell was viewed through the bottom of the chamber with an 100× oil immersion apoobjective lens and a 25× eye piece. The optical image was monitored and recorded with a video camera (Model DAGE-67 M; MTI Inc., Indiana) and a video cassette recorder system (model BR-S611U; JVC, Tokyo, Japan). The results were analyzed with a digital image-processing system (model RMC-D4; Brian Reece Scientific, Ltd., Berkshire, UK).

In response to the fMLP, the PMN deformed to crawl into the micropipette, and then moves steadily forward while filling the lumen of the micropipette. The application of a pressure via the micropipette to counter the chemotactic migration of the cell provided a direct measure of the force generation capacity of the cell.

All experiments were carried out at 25°C.

Address correspondence to Dr. Shu Chien, Institute for Biomedical Engineering, UCSD, La Jolla, CA 92093-0412.

RESULTS

As shown in Fig. 2, immediately behind the leading front edge of a locomoting PMN is a uniformly clear, gray zone (zone A) devoid of granules or other organelles, which probably is a region of actin gel. Immediately behind this clear zone is a short section (zone B) in which many granules undergo rapid, Brownian motions, suggesting that this region is quite fluid. The rear portion of the cell (zone C), containing the nucleus and organelles, shows little internal motion as the cell moves forward; it appears to be passively pulled forward due to the contraction of a cortical layer or of the bulk of the cytoplasm. The three zones are generally clearly discernible and tend to maintain their relative proportions as the cell moves forward. The total length of the cell is constant within a few percent as the cell moves. A sequence of photographs taken at different stages of the active motion of a PMN entering a micropipette is shown in Fig. 3.

When no pressure difference is applied across the cell, it moves steadily forward with an average velocity of about 0.33 μm/s, although the velocity is not constant. When a positive counter-pressure is applied during this forward movement, the cell slows down. A sample record for a single cell is shown in Fig. 4. With increasing levels of counter-pressure, the velocity of cell motion decreases. At a counter-pressure of about 17 cm H_2O, the cell velocity drops to zero and even moves backward

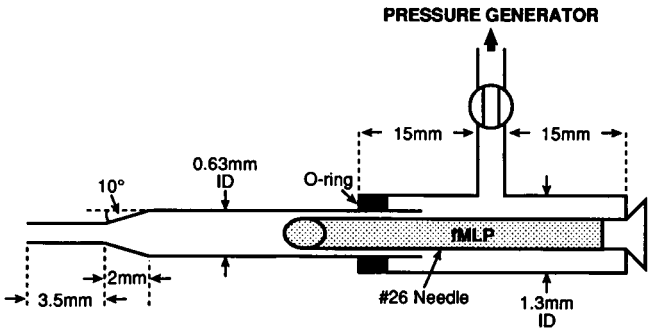

FIGURE 1 Schematic cross-section of the micropipette with the 26-gage needle containing fMLP. The dimensions are indicated in mm, but the drawing is not to scale.

with a bulging of its rear end. Similar results were obtained in 12 other cells. In some cases, the front end of the cell may remain adherent to the pipette wall, while the rest of the cell is elongated toward the rear in response to the counter-pressure such that the cell no longer fills the lumen. Therefore, the retreat process is not symmetrical to the advancement. The effects of counter-pressure on the cell velocity of 12 PMNs are summarized in Fig. 5. The PMN's velocity decreases linearly with increasing counter-pressure, and drops to zero at a counter-pressure of about 17 cm H_2O. These results indicate that the cell motile apparatus can generate a positive, forward thrusting pressure of at least 17 cm H_2O. Since the cell fills the lumen of the pipette at the level of optical microscopic resolution, the total force that the cell can generate is at least this pressure times the cross-sectional area of the pipette, yielding a value of ~0.003 dyn. It is interesting to note that the rate of work done by the cell (force · velocity) is maximum at the midpoint of the pressure-velocity line (7.5 cm H_2O, 0.17 μm/s) shown in Fig. 5. This maximum work rate is about 2.5×10^{-8} erg/s.

DISCUSSION

It is well established that most non-muscle cells have some ability to contract (e.g., in cell division). Harris et al. (1980) have demonstrated the wrinkling of an elastic substrate by cell contraction and derived an estimate of the contraction force of 0.001 dyn per μm of the advancing margin of a chick heart fibroblast. For a nominal width of 5 μm, this would yield a total force of 0.005 dyn. The thrusting forces measured in the present experiments are of a similar order of magnitude. Felder and

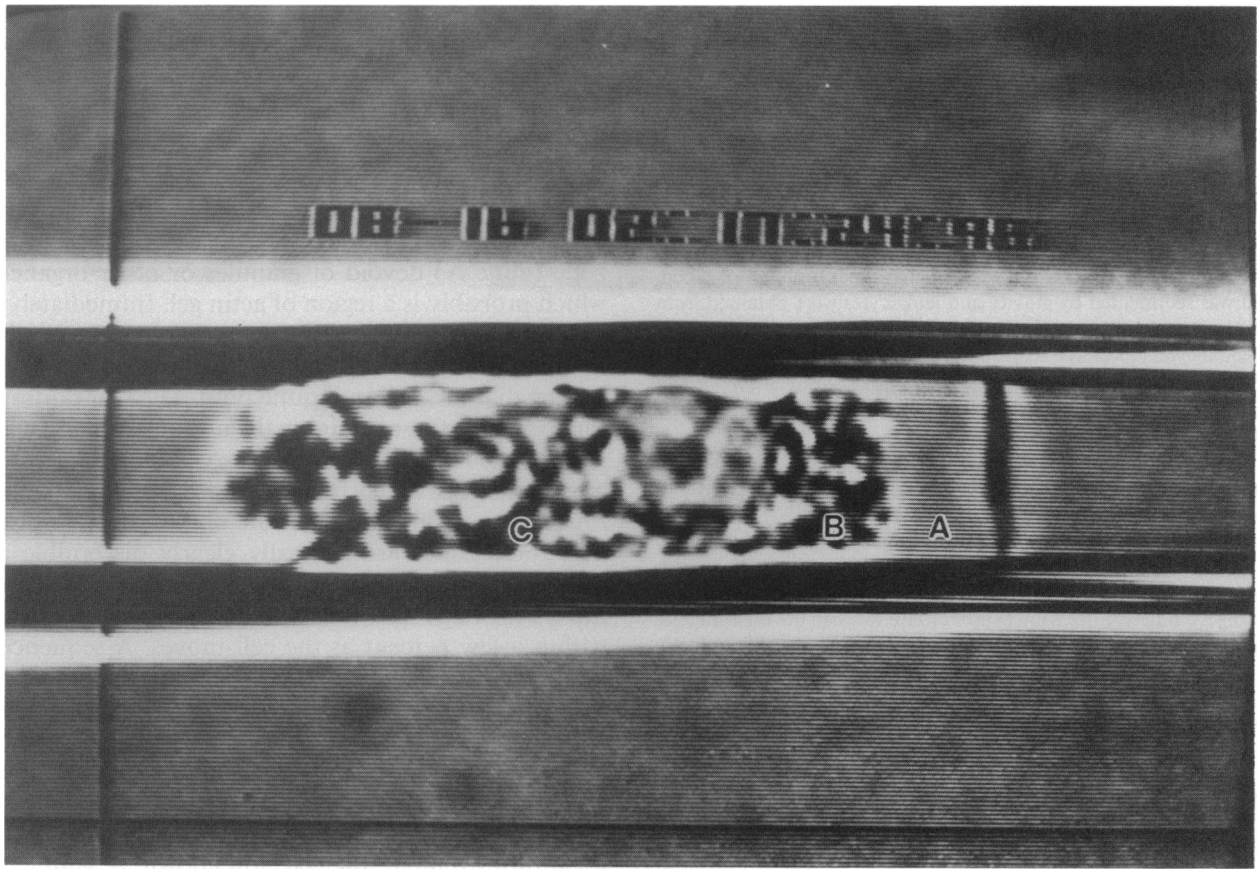

FIGURE 2 Videomicrograph of a PMN actively moving through a micropipette. The three zones (*A*, *B*, and *C*) labeled are generally clearly discernible and tend to maintain their relative proportions as the cell moves forward. The total length of the cell is constant within a few percent as the cell moves.

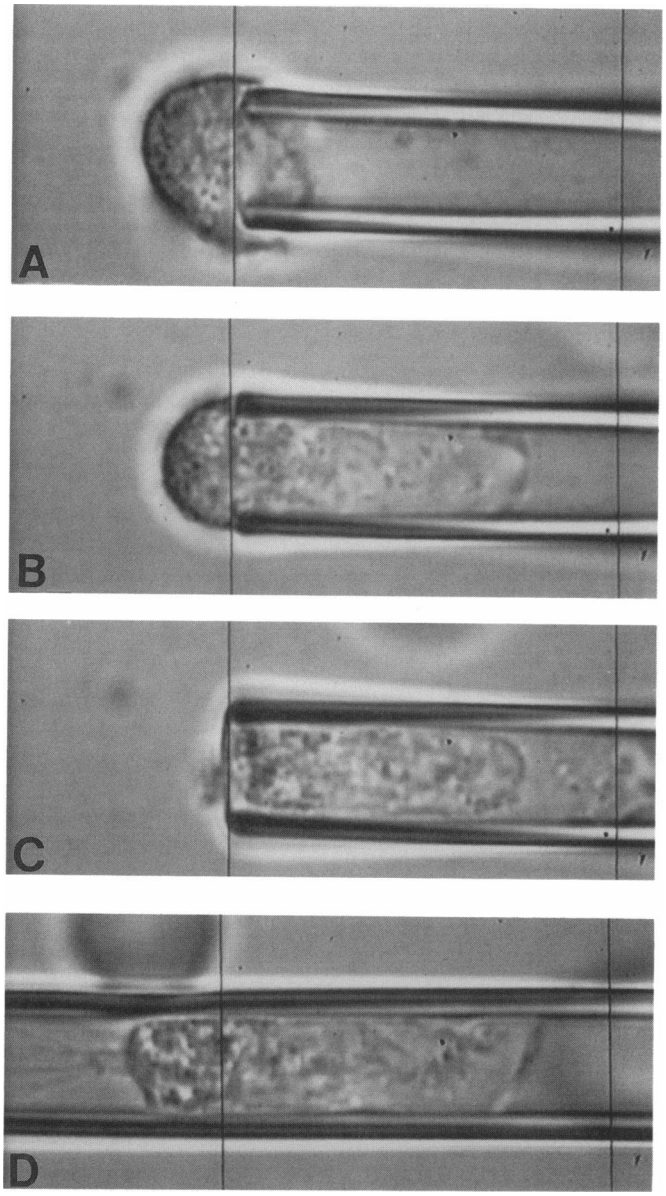

FIGURE 3 Composite photographs showing different stages of active motion of a PMN entering and moving through a micropipette. (*A*) Beginning of entry. (*B*) Nearly full entry. (*C*) Complete entry. (*D*) Steady motion in the micropipette. The microscope stage was kept stationary in *A*, *B*, and *C*; in *D* the image was shifted leftward in order to keep the moving cell in view.

Elson (1990), using a cell-poking technique, have determined the active force at the leading edge of lamellae of locomoting fibroblasts to be on the order to 5 μm-dyn per μm. The force measured was the retractive force rather than the extension force of protrusion, and the differences in the geometries of the lamella and the methods of measurement make it difficult to compare their results with that obtained in the present pipette tests.

Although the origin and the mode of transmission of the forward-thrusting force is not entirely revealed by the present experiments, it is clear that adhesion to the pipette wall occurs and is necessary to balance the forward thrust. The experiments with large counter-pressure show that the front end of the cell is tightly adherent to the pipette wall. On the other hand, at the rear part of the cell, adhesive bonds must be broken as the cell moves forward or as the cell is pushed back by counter-pressure.

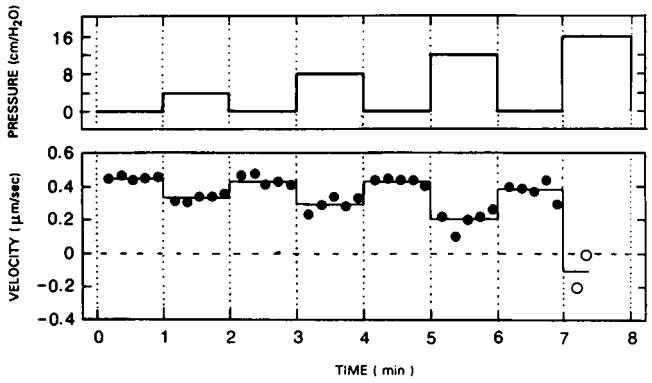

FIGURE 4 A sample data set derived from videotape recording on a single PMN. In this case, the micropipette (inner diameter 5.7 μm) was coated with fibronectin before the experiment. The vertical lines indicate the time at which the counter-pressure is changed. The top panel shows the time history of the counter-pressure applied. Each velocity point represents the mean determined over a 10-s interval (300 frames of video recording), and the horizontal lines denote the average velocities over the intervals between pressure changes.

The molecular mechanisms of the cell protrusion apparatus is not established. It is theoretically possible that contraction at the rear of the cell forces cytoplasm forwards, but it is also possible that the thrust is developed by the polymerization of actin at the leading edge membrane (Zhu and Skalak, 1988) or by osmotic pressure (Oster and Perelson, 1987; Stossel, 1990). In any case, the present measurements provide new quantitative information on the total force that a PMN can generate in response to a chemotactic stimulus and on the force-ve-

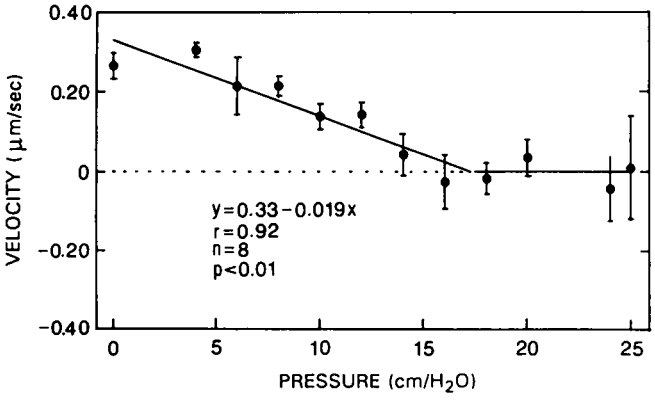

FIGURE 5 Summary of data for 12 PMNs showing the effects of changing counter-pressure on cell velocity. The data include cases in which the pipettes are coated and not coated with fibronectin. There is no statistically significant difference between these two cases. The line and equation shown in the figure are a least-squares fitting of the experimental data between pressure levels of 0 and 17 cm H_2O.

locity relation of the locomotor mechanism which may serve as a guide to a more complete resolution of the molecular mechanisms involved.

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REFERENCES

Felder, S., and E. L. Elson. 1990. Mechanics of fibroblast locomotion: analysis of forces and motions at the leading lamellas of fibroblasts. *J. Cell Biol.* 111:2513–2526.

Harris, A. K., P. Wild, and D. Stopak. 1980. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science (Wash. DC).* 208:177–179.

Heath, J. P., and B. F. Holifield. 1991. Cell locomotion: new research tests old idea on membrane and cytoskeletal flow. *Cell Motil. Cytoskeleton.* 18:177–179.

Holifield, B., A. Ishihara, J. Lee, and K. Jacobson. 1990. Movement of plasma membrane components during cell locomotion. *In* Optical Microscope for Biology. B. Herman and K. Jacobson, editors. Wiley-Liss Inc., New York. 473–486.

Oster, G. F., and A. S. Perelson. 1987. The physics of cell motility. *J. Cell Sci.* 8(suppl):35–54.

Stossel, T. P. 1990. How cells crawl. *Am. Sci.* 78:408–423.

Zhu, C., and R. Skalak. 1988. A continuum model of protrusion of pseudopod in leukocytes. *Biophys. J.* 54:1115–1137.