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# THE INTRACELLULAR DISTRIBUTION PATTERNS OF MYOSIN AND ACTIN ARE DIFFERENT AMONG HUMAN NEUTROPHILS AND MONOCYTES DURING LOCOMOTION

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**ABSTRACT**

**Background and Objective.** Neutrophils and monocytes initiate their characteristic ameboid movement by using mechanochemical systems of contractile proteins. It is known that neutrophils and monocytes exhibit differing patterns of motility. We set out to determine whether these differences may be associated with the intracellular distribution of myosin and actin, the principle components of the cellular apparatus involved in motility.

**Methods.** Myosin and F-actin in human neutrophils and monocytes were observed at resting and motile stages by using a double-fluorescence staining procedure and a confocal laser scanning microscope.

**Results.** In motile neutrophils, myosin was distributed in the lamellipodia and the cytoplasm, observed as a speckled pattern, whereas F-actin was concentrated in the front of the lamellipodia and in the perinuclear area. In the motile monocytes, myosin was found in the wide lamellipodia and was seen to radiate from the cytoplasm towards the edges of the cell in a punctate pattern. F-actin was densely distributed along the leading edge of the wide lamellipodia as well as in the perinuclear region. No differences were apparent in the intracellular distribution of myosin and F-actin between the resting neutrophils and monocytes.

**Interpretation and Conclusions.** Findings indicate that differing patterns of arrangement of myosin and actin in the lamellipodia and cytoplasm of neutrophils and monocytes may contribute to their movement, *in vitro*.


*Key words: neutrophil, monocyte, myosin, actin, lamellipodia*

The motility of non-muscle cells results from a mechanochemical system of contractile proteins.[1-6] The contractile proteins myosin, actin, and actin-associated proteins constitute the motile apparatus as well as the cytoskeleton of the cells.

Neutrophils and monocytes initiate their characteristic ameboid movement by producing mechanical energy derived from the hydrolysis of adenosine triphosphate (ATP), similar to the contraction of muscle, which involves myosin and actin. The locomotion and phagocytosis of leukocytes and monocytes are based on the activities of the lamellipodia. These activities permit the neutrophils to move at a speed of 12 μm/min to –40 μm/min, while monocytes move at a speed of approximately 10 μm/min.[1,3] Scanning electron microscopy observations have shown that during locomotion on a glass slide, neutrophils extend thick, cauliflower-like lamellipodia, while monocytes, with their strong adhesive property, extend flat and spreading lamellipodia.[3,7]

We previously purified $Ca^{2+}$-sensitive contractile proteins from equine neutrophils and human leukemia cells that were similar to muscular contractile proteins.[8-14] However, in contrast to studies done in fibroblasts,[15,16] *Acanthamoeba* and *Dictyostelium*,[17-21] the morphological and immunocytochemical study of myosin and actin in human neutrophils and monocytes was hampered by the complications involved in trying to visualize such small cells by conventional microscopy. Also, it is difficult to preserve native myosin and actin in these cells during preparation for their study by light and transmission electron microscopy, and electron microscopy images of native myosin and actin in human leukocytes have not been published to date.

We recently reported the localization of myosin and actin in human neutrophils and leukemia cells during movement by using a conventional fluorescence microscope and a single-fluorescence staining procedure.[22] We also developed a double-fluorescence staining procedure through which the relative distribution patterns of myosin and actin in a single neutrophil could be simultaneously observed during locomotion by use of conventional fluorescence microscopy.[23] In the preparation of specimens we

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used a purified anti-myosin antibody[22] and phalloidin, which stains filamentous actin (F-actin).

We then applied this double-fluorescence staining procedure to simultaneously observe the distribution pattern of myosin and actin in the same leukocyte by using a fluorescence microscope[24] or a confocal laser microscope. This was done to determine whether differences in the motility rate, the formation of lamellipodia, and the ability of neutrophils and monocytes to adhere to the surface of a glass slide may be associated with the specific intracellular arrangement of myosin and actin in these cells.

## Materials and Methods

*Cell preparation*

Two mL of venous blood was withdrawn from a healthy young volunteer and anticoagulated with a tenth volume of 3.8% sodium citrate. This blood sample was then centrifuged through a Percoll (Sigma Chemical Co.) density gradient at 90×g for 10 min. The separated neutrophils and monocytes were subsequently suspended in 10 mL of phosphate-buffered saline (PBS) pH 7.4, and used immediately in the following studies of cell motility.

*Fixation protocols*

A 200 µL suspension of neutrophils or monocytes in PBS that contained 5.5 mM glucose was placed at room temperature on a glass slide cleaned with ethanol.

Leukocytes suspended at 4°C were immediately fixed in 1% formaldehyde in PBS in resting state and incubated for 20 min in a moist chamber at room temperature.

Leukocytes suspended at room temperature were incubated in a moist chamber at 37°C for 20 min to allow the cells to become motile. After the actively motile adherent cells had been visualized by phase-contrast microscopy, they were fixed in 1% formaldehyde in PBS and incubated for 20 min in a moist chamber at room temperature. The fixed cells were then washed in PBS and post-fixed in 0.1% glutaraldehyde in PBS for 20 min in a moist chamber at 4°C. To remove the additional formaldehyde and glutaraldehyde from the fixed cells, the preparations were washed three times in 500 mL PBS that contained 0.05% Tween 20. The washings were done for 20-min intervals at 4°C. These washed cells were used to study the relative distribution of myosin and actin during a state of adherent motility.

*Dual staining procedures*

Fixed cells (neutrophils or monocytes) were pretreated for 30 min with 5% goat serum containing 1% bovine serum albumin (BSA) in a moist chamber at room temperature. To remove the additional goat serum and BSA in the fixed cells, the preparations were washed in 500 mL PBS containing 0.05% Tween 20 for 30 min at 4°C. The postfixed cells were then reacted with an anti-myosin antibody (diluted 1:50) that was produced as previously described.[22,25,26] This antibody reaction was performed in PBS containing 1% BSA incubated overnight at 4°C in a moist chamber. In order to remove unbound antibody after this incubation, the cells were washed in 500 mL PBS containing 0.05% Tween 20 for 30 min at 4°C. Subsequently, this preparation was reacted with a fluorescein isothiocyanate (FITC)-conjugated affinity-purified goat anti-rabbit IgG (diluted 1:50; Jackson ImmunoResearch Laboratories, Inc., PA, USA). This reaction was carried out in PBS containing 1% BSA and was incubated at 37°C for one hour in a dark, moist chamber. After two 30-min washes at 4°C in 500 mL PBS containing 0.05% Tween 20 to remove any excess FITC-conjugated antibody in postreacted cells, the cells were treated with rhodamine-conjugated phalloidin (diluted 1:10; Molecular Probes, Inc. Eugene, OR, USA) at room temperature for 30 min in a dark, moist chamber. The excess phalloidin was then removed by washing the cells in 500 mL PBS containing 0.05% Tween 20 for 30 min at 4°C. The glass coverslips were then mounted with 90% glycerol in PBS and the periphery of the coverslips was sealed with nail polish.

*Microscopy*

A BioRad MRC-1024 confocal imaging sytem microscope (BioRad Labs, Hemel Hemstead, UK) fitted with Krypton/Argon (488/568nm) lasers for confocal microscopy, and an interference reflection microscope were used. Confocal laser microscopic images and interference reflection microscopic images of cells were obtained. Images were acquired by using an oil immersion 60x plane apo objective (Nikon, Japan).

## Results

*Myosin and actin in resting neutrophils*

In the resting stage, the neutrophils exhibited a spherical form on a glass slide (Figure 1D), and a homogeneous green fluorescence (Figure 1A). Red fluorescence was also observed in these neutrophils in a pattern similar to that seen for the green fluorescence (Figure 1B). The whole body of resting neutrophils showed the homogenous yellow fluorescence in a dual image (Figure 1C). These results suggest that both myosin and F-actin coexisted uniformly in the entire cytoplasm of the resting neutrophils.

*Myosin and actin in motile neutrophils*

When the neutrophils were warmed at 37°C on a microscopic stage, they began to move, forming thick lamellipodia while adhering to the slide (Figure 2D). Myosin was densely distributed throughout the

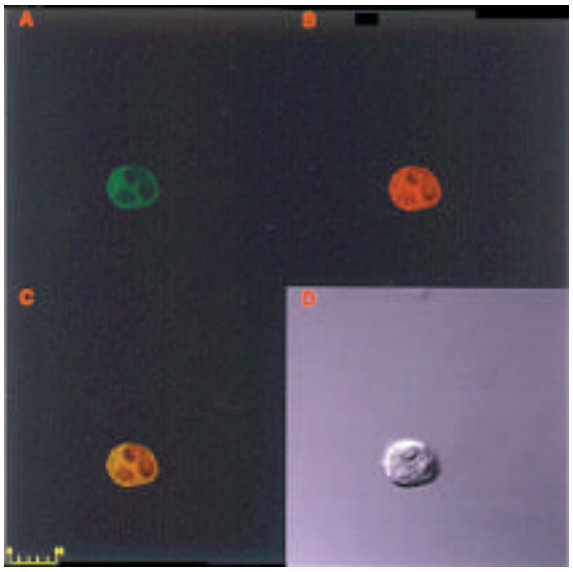


Figure 1. FITC staining of myosin and rhodamine staining of F-actin in a resting neutrophil. A resting neutrophil was reacted with the following: anti-myosin antibody, FITC-labelled anti-goat anti-rabbit IgG, and then with rhodamine-labelled phalloidin as described in *Materials and Methods* section. This neutrophil was visualized by immunofluorescence microscopy (A, FITC; B, rhodamine), dual immunofluorescence microscopy (C), and interference reflection microscopy (D).

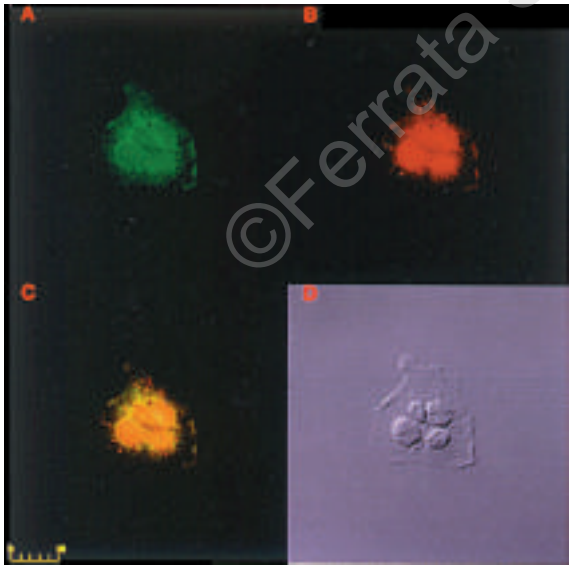


Figure 2. FITC staining of myosin and rhodamine staining of F-actin in a motile neutrophil. A motile neutrophil was reacted as in Figure 1. Microphotographs obtained by immunofluorescence microscopy (A, FITC; B, rhodamine), dual immunofluorescence microscopy (C), and interference reflection microscopy (D) of the same neutrophil are presented.

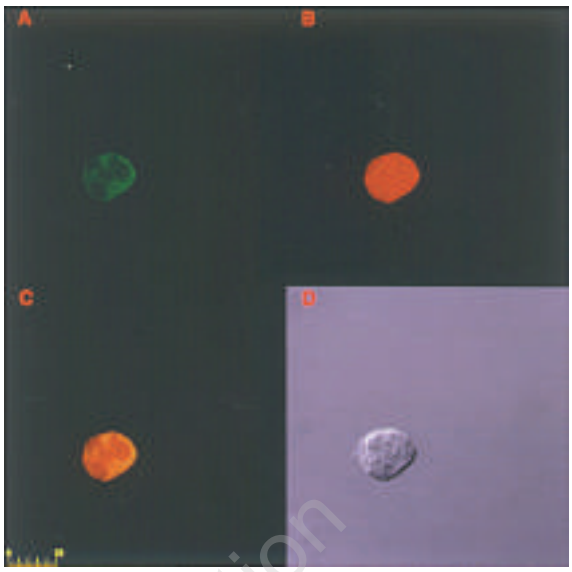


Figure 3. FITC staining of myosin and rhodamine staining of F-actin in a resting monocyte. A resting monocyte was reacted as in Figure 1. This monocyte was then visualized by immunofluorescence microscopy (A, FITC; B, rhodamine), dual immunofluorescence microscopy (C), and interference reflection microscopy (D).

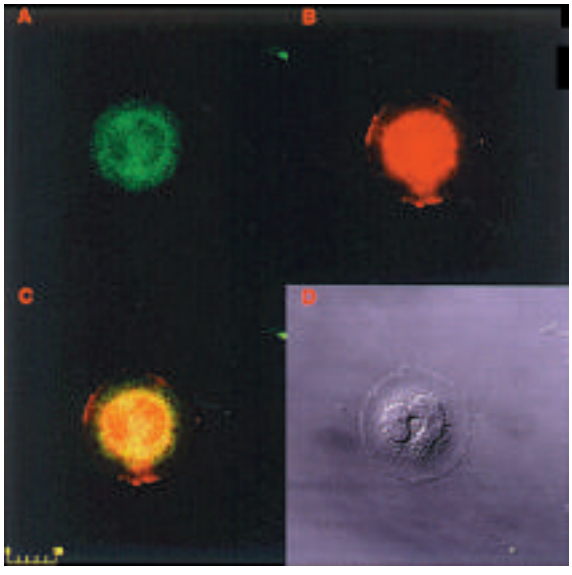


Figure 4. FITC staining of myosin and rhodamine staining of F-actin in a motile monocyte. A motile monocyte was reacted as in Figure 1. Images of the same monocyte are presented as viewed by the following: immunofluorescence microscopy (A, FITC; B, rhodamine), dual immunofluorescence microscopy (C), and interference reflection microscopy (D).

lamellipodia and the cytoplasm of these cells, with approximately ten myosin rods displaying a speckled pattern. Additional myosin fluorescence was also seen in the perinuclear region and in the areas that surrounded the granulomere (Figure 2A). F-actin was concentrated in the front of the lamellipodia during movement. In addition, F-actin was distributed in the perinuclear region, while only weak fluorescence for F-actin was observed in the remaining portion of the cytoplasm (Figure 2B). The lamellipodia showed the yellow fluorescence, the cytoplasm showed the speckled green fluorescence, and the perinuclear region showed the yellow fluorescence in a dual image (Figure 2C). These results suggest that the level of F-actin in the cytoplasm during locomotion is minimal.

*Myosin and actin in resting monocytes*

At rest, the monocytes exhibited a spherical form and were slightly larger than the neutrophils on a glass slide (Figure 3D). Patterns of green, red, and yellow fluorescence similar to the fluorescence distributions seen in the round neutrophils were observed in the monocytes (Figures 3A, 3B, 3C). These results show that both myosin and F-actin were diffusely distributed throughout the body of the resting monocytes, similar to findings with the resting neutrophils.

*Myosin and actin in motile monocytes*

The monocytes incubated at 37°C began to move as they formed thin, spreading lamellipodia (Figure 4D). These lamellipodia were seen as large protrusions of the main cell body, presumably due to the highly adhesive nature of these cells on the glass slides. As shown in Figure 4A, radial extensions of myosin fluorescence were seen as a dotted pattern that began at the granulomere and extended in the direction of the cell membrane in the lamellipodia. Additional myosin-derived fluorescence was seen in the perinuclear region as well as around the granulomere (Figure 4A). F-actin was concentrated at the leading edge of the lamellipodia during locomotion, while the remainder of the F-actin was densely distributed in the perinuclear region, resulting in a weak fluorescence intensity in the cytoplasm and around the granulomere (Figure 4B). The lamellipodia showed the red fluorescence, the cytoplasm showed the dotted green fluorescence, and the perinuclear region showed the yellow fluorescence in a dual image (Figure 4C). These observations suggest that the cytoplasmic content of F-actin was reduced during locomotion, as was seen in the neutrophils. The F-actin structural network appeared to be intact in the lamellipodia.

## *Discussion*

The forces that drive the movement of the leukocytes result from the mechanochemical energy released by the hydrolysis of ATP when myosin-actin contractile interactions take place.[1-6,27-30] Neutrophils and monocytes initiate movement in response to a variety of external stimuli. These cells form lamellipodia, move about, and phagocytose particulates. Therefore, an analysis of the activity of the lamellipodia is required to elucidate the mechanism of the leukocyte locomotion. Although similar events take place, the shape of the lamellipodia differs between the neutrophils and monocytes, as does the speed of movement and the adhesiveness of the cell. We observed no apparent differences in the biochemical and morphological properties of myosin and actin between the neutrophils and monocytes. It is thought that the majority of the myosin in the leukocytes is in the monomeric state and is present in the cytoplasm, as actin molecules in the non-filamentous state are unbound to actin molecules in the resting state. It is also believed that myosin is mainly converted to oligomers, causing the actin molecules to become filamentous, and that myosin interacts with F-actin during cellular movement. We therefore investigated the relative distribution patterns of myosin and actin from the morphological viewpoint by observing the motile apparatus of the cell during locomotion with fluorescence microscopy and immunofluorescence procedures. We found that the patterns of intracellular distribution of myosin and actin differed between neutrophils and monocytes during locomotion, although there was no apparent difference in the relative distribution of myosin and F-actin in the resting cells.

Our results indicate that the reconstitution of myosin and actin in the lamellipodia differs between neutrophils and monocytes during movement. These findings may account for the differences in the formation of the lamellipodia, the rate of motility, and the varied adhesive properties of these cells.

However, a force-generating mechanism between myosin and actin in these cells is unclear. Furthermore, how actin-associated protein interacts with myosin and actin during motion of the leucocytes and in the formation of lamellipodia is also unknown. We suggest that the actin network attached to the cell membrane via actin-associated protein is constructed when the actin is reorganized and polymerized in the leading portion of the leukocytes in response to a stimulus. This produces tension and the formation of lamellipodia when molecules of myosin slide on the F-actin cable networks that are bundled or cross-linked by actin-associated protein or myosin oligomers attached to the cell membrane. The sliding of myosin molecules over the polymerized actin causes the back of the leukocyte to be pulled along, and as a result, the body of the leukocyte moves forward.[31]

Recently, the myosin isoenzymes, myosin I and

myosin II, which are found in *Acanthamoebae*, *Dictyostelium* amebae, and fibroblasts (among other cells), have been implicated in the mechanism of contraction. The intracellular location of these isozymes has been observed by fluorescence microscopy using an immunofluorescent staining procedure[15,19,21,32,33,34] and their functions and characteristics have been elucidated.[35-38] It was reported that the direction and speed of migration of ameboid cells that lack functional myosin II differ from those observed in wild-type cells.[39] In human leukocytes, other myosins, excluding myosin II, have recently been reported, suggesting that additional levels of molecular complexity are involved in the process of locomotion.[36,40] Further studies of contractile proteins that contain myosin isoenzymes are required to elucidate the mechanism of leukocyte movement, and may help in clarifying the differences between motile neutrophils and monocytes with respect to the shape of their lamellipodia, the speed of their movement, and their adhesiveness.

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