ASSOCIATION OF MICROFILAMENT BUNDLES WITH LYSOSOMES IN POLYMORPHONUCLEAR LEUKOCYTES

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It is well known that polymorphonuclear (PMN) leukocytes move and that lysosomes move within these cells (21). It has been suggested that microtubules or microfilaments are involved in these movements (1, 17, 22). This paper demonstrates that microfilaments are associated with lysosomes and suggests that organized contractile microfilaments are involved in lysosomal movement.

Much of the evidence indicating that microtubules or microfilaments are involved in PMN leukocyte movement is indirect evidence. These studies used colchicine or cytochalasin B to stop movements. Although these drugs stop movement, the mechanism by which they work on living cells is not clear; they may act on microtubules, microfilaments, membranes, or any combination of these sites. For example, colchicine not only depolymerizes microtubules (5) but also binds to membranes (48) and produces structural changes within plasma membranes (16). Thus, the interpretation that colchicine stops lysosomal movements solely because it binds to microtubular proteins may not be entirely correct.

Similarly, the inhibition of movement by cytochalasin B does not directly demonstrate the involvement of contractile filaments. Cytochalasin B inhibits membrane transport and supresses glycolysis (54, 55). At high concentrations, it inhibits leukocyte phagocytosis (7, 11, 30), exocytosis (11), and chemotaxis (3) while at low concentrations it stimulates these processes. Thus, the mechanism of cytochalasin B is more complex than was initially suspected (8, 13), and there still appears to be a controversy as to its site of action (15, 47).

Current biochemical research on microtubules and contractile filaments in nonmuscle cells has provided a clearer insight into the possible cytoplasmic roles of these structures. The depolymerization and repolymerization of microtubules in vitro (38, 44, 52) supports the notion that microtubules play an important role in cell shape changes by assembling and disassembling in vivo. Recent antibody studies with monospecific antitubulin further support the thinking that microtubule polymerization occurs in vivo (39). The regulation of microtubule polymerization may depend on several cytoplasmic factors including ionic environment (52), cyclic nucleotides (53) and microtubule-associated proteins (12). At present, there is no direct evidence that cytoplasmic microtubules produce the motive force in cytoplasmic movement; evidence is lacking that an active dynein ATPase is associated with cytoplasmic microtubules. Thus, it is only clear that microtubule assembly is important for cell shape transformations.

Studies on microfilaments in the demembranated cytoplasm of the free-living amoeba have clearly indicated that calcium-activated contractile filaments can interact to produce movements in vitro in the absence of microtubules (50). The recent biochemical isolation and identification of actin and myosin filaments in polymorphonuclear leukocytes suggests that an actomyosin contractile system could be responsible for leukocyte movement (49).

In this paper, we report the close association of organized bundles of microfilaments and short "filaments" between lysosomes and microfilament bundles. Although skeins of microfilaments have been reported in PMN leukocytes (30), to the best of our knowledge this is the first report of the

association of filament bundles with lysosomes in mammalian leukocytes. Our observations suggest that organized contractile filaments may play a role in lysosomal movements within the cell.

MATERIALS AND METHODS

Cell Isolation and Fixation

Rabbit PMN leukocytes were obtained from peritoneal exudates induced by injection of glycogen (1 mg/ml) in a phosphate buffer solution (pH 7.2–7.4) 4 h before harvesting the cells (20). After isolation, motile cells were resuspended in Eagle's minimal medium (final concentration of 2×10^6 cells/ml) at 37°C. Cells were fixed for 1 h with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, 380 ± 20 mOsm. Fixed cells were pelleted and stored overnight in 7% sucrose in 0.1 M cacodylate buffer pH 7.4, 380 ± 20 mOsm. The cells were postfixed for 1 h in 2% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4. With this fixation procedure, no visible change in cell shape or structure was observed in the light microscope or the scanning electron microscope (34, footnote 1).

Transmission Electron Microscopy

For transmission electron microscopy, the cells were dehydrated in ethanol, embedded and cured in Epon 812 (29) The blocks were sectioned with an LKB-ultramicrotome (LKB Instruments, Inc., Rockville, Md.). Thin sections were picked up on uncoated copper grids and stained with uranyl acetate and lead citrate (43). Electron micrographs of thin sections were taken on a Hitachi HU-12 electron microscope operated at 75 kV.

Scanning Electron Microscopy

Leukocytes were prepared for scanning electron microscopy as described elsewhere (footnote 1). Briefly, the cells were isolated, fixed with glutaraldehyde and osmium tetroxide (see above), treated with thiohydracarbazide and osmium tetroxide (27), dehydrated in ethanol, cryofractured (19), critically point dried (2, 9), and coated with a thin (ca. 100 Å) conducting layer of gold or gold palladium. The cells were then observed in a Coates and Welter Model no. 106 scanning electron microscope operated at 16 kV.

RESULTS

Transmission Electron Microscopy

Microfilaments generally were found in four locations within PMN leukocytes: (*a*) as single filaments near the plasma membrane (Fig. 1), (*b*) as single filaments near the lysosomes (Fig. 2), (*c*) as single filaments approximately parallel to cytoplasmic microtubules (Fig. 3), and (*d*) within bundles of filaments in the cytoplasm (Fig. 4).

In thin-sectioned cells, bundles of microfilaments appeared to be associated with lysosomes (Fig. 4). The filamentous bundles measured about 0.1 μm in diameter and at least 1.0 μm in length. The diameters of the individual filaments ranged from 5 to 7 nm, which is similar to that observed for muscle actin filaments (23). Within the bundles the center-to-center distance separating ap-

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FIGURE 1 This electron micrograph shows a single microfilament (*f*) near the plasma membrane (*M*) of a rabbit polymorphonuclear leukocyte. Bar is 0.1 μm. × 54,000.

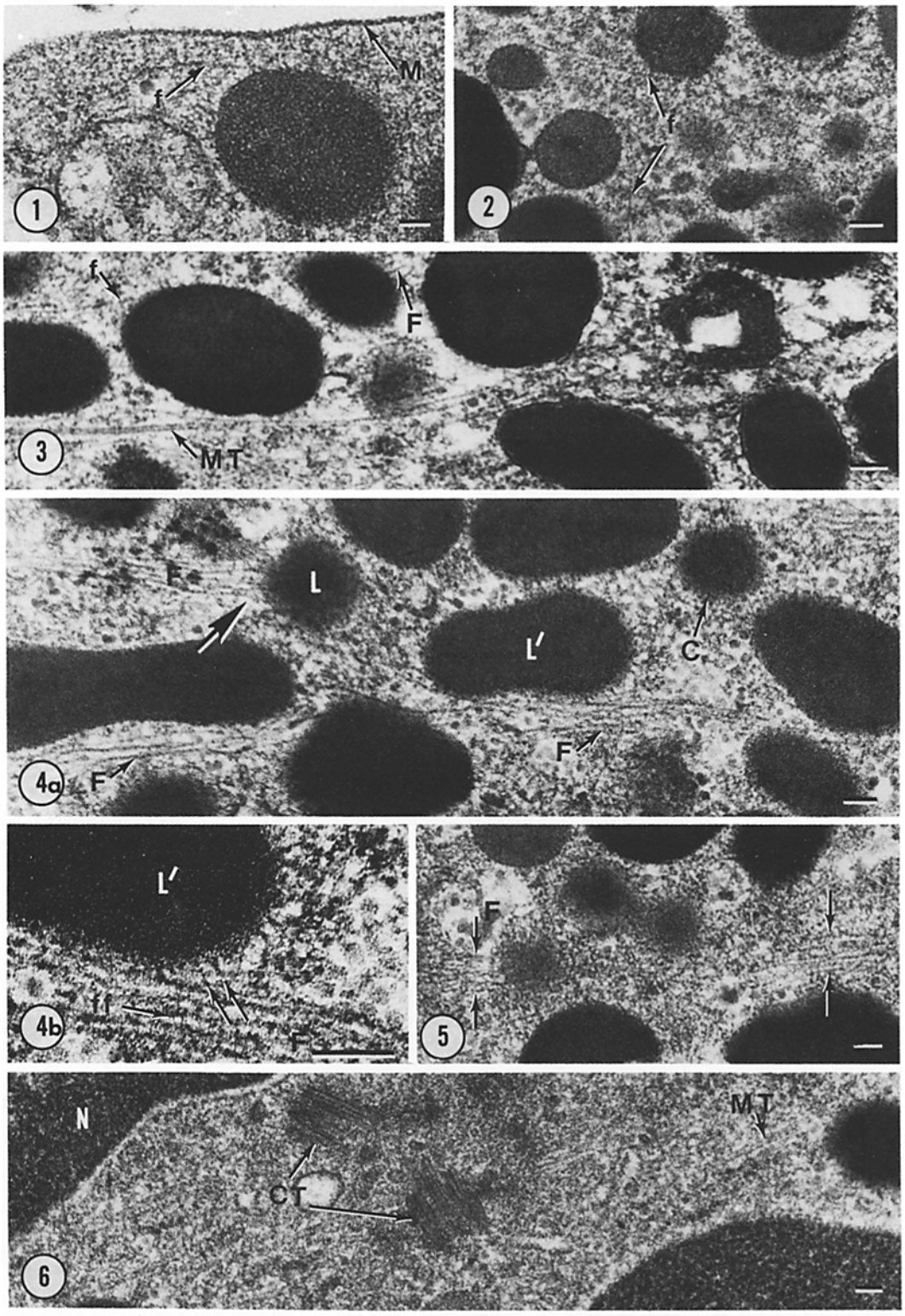
FIGURE 2 This electron micrograph shows single filaments (*f*) in the vicinity of lysosomes. Bar is 0.1 μm. × 54,000.

FIGURE 3 This figure shows filaments (*f*) which are approximately parallel to the cytoplasmic microtubules (*MT*). Note the bundle of filaments (*F*) in close association with the lysosomes. Bar is 0.1 μm. × 61,500.

FIGURE 4 (*a*) In fortuitous thin sections, bundles of microfilaments (*F*) are closely associated with lysosomes (*L,L'*) (large arrow). The lysosomes appear to have a "fuzzy coat" (*C*) on the cytoplasmic surfaces. (*b*) This enlargement of Fig. 4*a* shows the lysosome (*L'*) and its associated microfilament bundle (*F*). Short filamentous structures (2 black and white arrows) can be seen between the microfilament bundle and the lysosome. In addition, very fine filaments (*ff*) can be seen between microfilaments within the microfilament bundle. Bar in both figures is 0.1 μm. (*a*) × 54,000; (*b*) × 130,000.

FIGURE 5 Frequently, numerous glycogen granules obscure the filament bundles (*F*, see the region between the arrows). Bar is 0.1 μm. × 49,200.

FIGURE 6 This figure shows two centrioles (*CT*) and cytoplasmic microtubules (*MT*) near the nucleus (*N*). Bar is 0.1 μm. × 45,000.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proximately parallel filaments was about 15 nm. Microfilament bundles observed in different cells were closely associated with lysosomes (Figs. 3 and 4). Very fine filaments were seen between individual microfilaments within the bundle (Fig. 4*b*). Frequently, the filament bundles were obscured by the numerous glycogen granules in the cytoplasm (Fig. 5). In addition, short filamentous structures were seen between the lysosomes and the microfilament bundles. These short filaments were 10–30 nm in length and 2–9 nm in diameter (Fig. 4*b*). Similar short filaments were not observed between microtubules and lysosomes, or between microtubules and mitochondria as seen in neurons by others (46).

Centrioles and microtubules, similar to those reported previously (4, 22), were observed. The centrioles were frequently found in the vicinity of the nucleus, and the microtubules were found both near the centrioles and in the surrounding cytoplasm (Fig. 6).

Scanning Electron Microscopy

In cryofractured leukocytes a similar close arrangement of fibrous bundles and lysosomes was observed (Fig. 7*a,b*). The diameter of these bundles was similar to the diameter of the filamentous bundles seen in thin section.

DISCUSSION

Our results show a close association between filament bundles and lysosomes in PMN leukocytes. The microfilament bundles appear to consist primarily of actin-like filaments since the diameter of the filaments is similar to that of muscle actin filaments (23). We are currently attempting heavy meromyosin binding experiments (26, 41) in an effort to confirm that they are actin filaments. However, Stossel and Pollard have demonstrated the presence of an actomyosin system in guinea pig PMN leukocytes (49), and Boxer and Stossel have demonstrated actin in human PMN leukocytes (6). Thus, it seems likely that rabbit PMN leukocytes also contain actin and myosin.

The longitudinal organization of contractile filaments in muscle is considered to be an important prerequisite for the sliding filament theory (24, 25). The presence of longitudinally organized microfilaments in intact PMN leukocytes suggests that a similar organization of contractile filaments exists in these cells. In their size, length and branching patterns these microfilament bundles are similar to microfilament bundles seen in amoeba cytoplasm (31, 33). Recent advances in the study of cytoplasmic streaming in plant cells have yielded important information on the organization of contractile elements in the cytoplasm. F-actin filaments have been identified in filament bundles (10, 40). In *Nitella* the filament bundles are located at the ecto-endoplasmic interface, and the filaments are organized so that the arrowheads of all the actins point in the same direction (40), a direction which is opposite to the direction of streaming (28).

Our observations of an association between microfilament bundles and lysosomes in leukocytes resembles the association seen between filament bundles and chloroplasts in glycerinated models of *Nitella* (40). The association also resembles that seen between filament bundles and pigment granules in glycerinated retinal pigment epithelial cells of the frog (36). However, our observations differ from previous observations since our cells were not glycerinated and since occasional short filaments were seen between the microfilament bundles and lysosomes. We believe this to be the first report of definitive structures between filament bundles and cytoplasmic organelles. These short filaments appear to be structurally similar to those between bundles of actin filaments and the plasma membrane in the microvilli of intestinal epithelial cells (35).

The morphological observation of a close association of filament bundles with lysosomes and of short filaments between these structures suggests that binding proteins might exist between filaments and lysosomes. The recent isolations of actin-binding proteins such as actin-binding protein in macrophages (18), alpha-actinin (45), and spectrin (51) provide evidence that actin-binding proteins are present in nonmuscle cells. It is conceivable that one or more of these proteins is associated with lysosomal membranes and that they might occur as short filaments.

Recent evidence indicates that lysosomal membranes in PMN leukocytes also contain intramembranous particles (34, footnote 1), glycoproteins associated with their membranes (37) and carbohydrate moieties on their cytoplasmic surfaces (14). The structural and functional relationships between carbohydrate components, lysosomal intramembranous particles, and the short filaments seen in thin sections remain to be fully elucidated.

It is hypothesized here that the short filaments

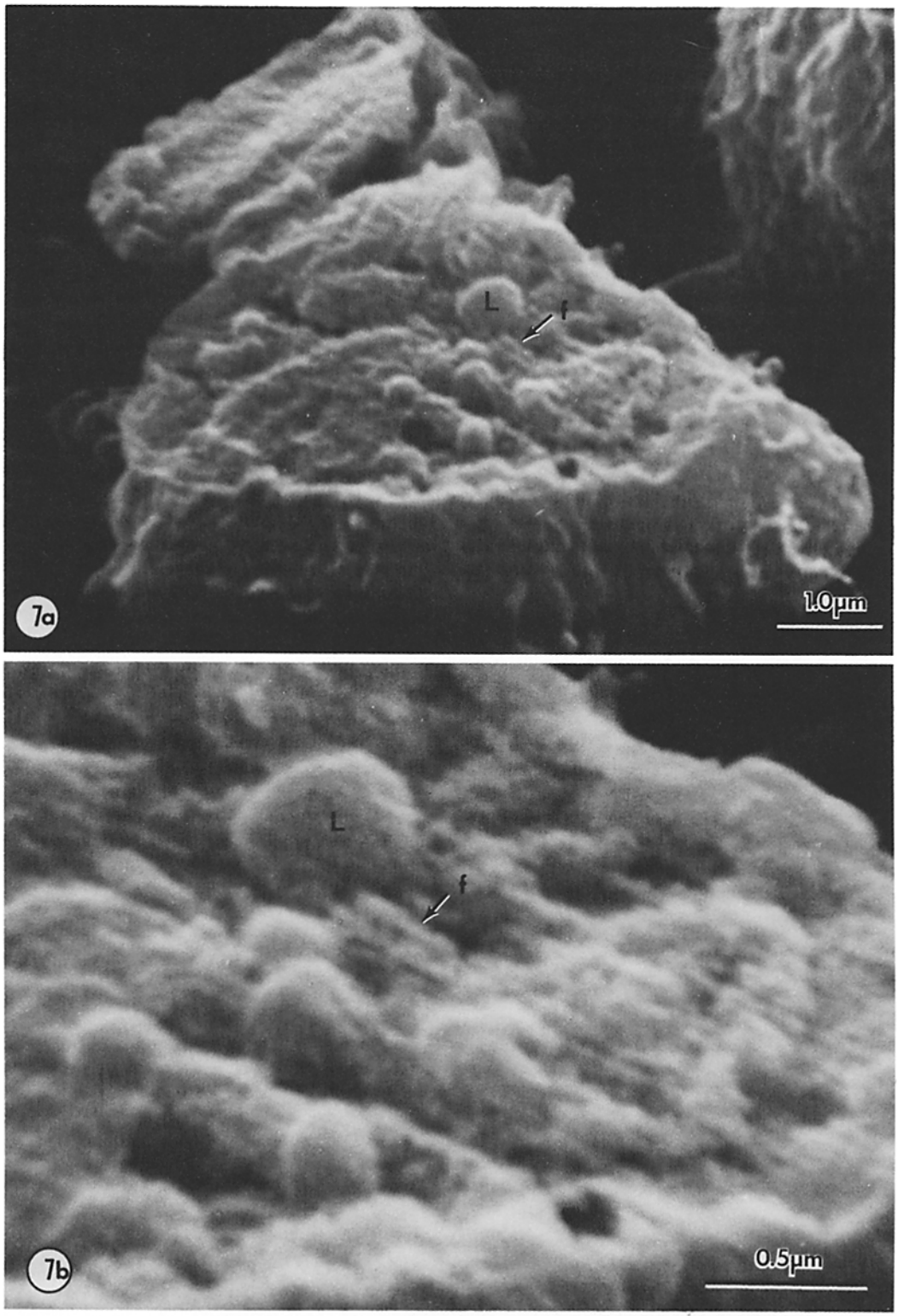

FIGURE 7 These scanning electron micrographs show a cryofractured rabbit PMN leukocyte. An arrangement of fibrous bundles (*f*) and lysosomes (*L*) similar to that observed in thin sections can be seen. (*a*) Bar is 0.1 μm. × 17,700. (*b*) Bar is 0.5 μm. × 50,000.

represent attachment sites between the lysosome and contractile filaments. If such attachment sites exist, it is conceivable that they are involved in lysosomal movement and that actin attached to membranes and interacting with myosin produces cytoplasmic movements. Such an explanation, if true, could be used to explain the sudden translocation of cytoplasmic particles known as saltatory movements. These types of movements are known to occur in a wide variety of cells (42).

It is not clear why a structural association between lysosomes and microfilament bundles has not been reported previously. Several explanations seem likely. The diameter of the filament bundles is small (about 0.1 μm), and the bundles are not distributed uniformly throughout the cytoplasm. In addition, the filament bundles are frequently obscured by other cytoplasmic structures such as glycogen granules (Fig. 5). This makes them difficult to visualize in thin sections, especially in the vicinity of the lysosomes. Finally, any condition which might disrupt the lysosomes such as hypo- or hyper-osmotic fixatives might also cause the disruption of filament-lysosome associations.

Since PMN leukocytes, like many other mammalian cells, contain both microtubules and microfilaments, it is difficult to assess the individual roles of these structures in cytoplasmic movements. At present, there is little evidence that cytoplasmic microtubules produce movements by themselves. Instead, current biochemical evidence suggests a dynamic cytoskeletal function for cytoplasmic microtubules since they can be polymerized and depolymerized in vitro (44, 52). Lysosomes may indeed require intact microtubules for movement. In fact, the changes in cell shape that occur during chemotaxis and emigration might be facilitated by microtubular reorganization. But, the possible attachment of actin filaments to the lysosomes suggests that an actomyosin-like system may be providing the motive force for the movement of lysosomes. That is, microtubules might delineate the "tracks" along which the lysosomes are moved. There is now good evidence that interactions between actin and myosin microfilaments produce cytoplasmic movements in nonmuscle cells (50). Hence, it seems likely that organized filaments may be involved in lysosomal movements in leukocytes. We also have noted similar microfilament bundles near phagocytic vacuoles (footnote 1). These filaments may be responsible for the limited alignment of intramembranous particles seen in freeze-fractured phagocytic vacuole membranes and may also be involved in degranulation.

SUMMARY

The juxtaposition of microfilament bundles and lysosomes seen both in thin-sectioned cells in the transmission electron microscope and in cryofractured cells in the scanning electron microscope, and the presence of short filamentous structures between lysosomes and microfilament bundles, suggest that microfilaments may be attached to lysosomal membranes and that these filaments may be involved in lysosomal movements. Further work is in progress to test these hypotheses.

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