

Brief Definitive Report**Colocalization of F-actin and Talin during Fc Receptor-mediated Phagocytosis in Mouse Macrophages**

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Summary

We have studied the distribution of talin in J774 cells and mouse peritoneal macrophages undergoing Fc receptor-mediated phagocytosis. At early stages of phagocytosis, talin accumulates in the cells' cortical cytoplasm adjacent to the forming phagosome and extends into pseudopods that are encircling the particle. Talin colocalizes with F-actin at these sites. After particle ingestion is completed, F-actin and talin are no longer concentrated adjacent to phagosomes. Thus, talin and F-actin undergo dynamic and coordinate changes in their cytoplasmic location during Fc receptor-mediated phagocytosis.

Engulfment of ligand-coated particles by leukocytes requires communication between ligand-activated plasma membrane receptors and the actin-based cytoskeleton. For Fc receptor-mediated phagocytosis, the precise signals that accomplish this are unknown. F-actin is recruited to the region of cell membrane in contact with the particle during engulfment (1, 2), and cytochalasins block both F-actin assembly (3) and particle engulfment (4, 5). These data suggest that F-actin assembly plays a major role in phagocytosis. Whether the newly assembled F-actin interacts directly with the segments of plasma membrane beneath the forming phagosome is unknown. One system that has been used to explore the molecular basis of interactions between membrane proteins and the actin-based cytoskeleton is the fibroblast adhesion plaque (6). When fibroblasts settle onto surfaces coated with extracellular matrix proteins (e.g., fibronectin), they bind to these surfaces via specific transmembrane receptor proteins called integrins. Integrins provide transmembrane linkages between extracellular matrix proteins and the cytoskeleton (7). Talin is a 225-kD cytosolic protein that is recruited to sites where integrins bind to the cytoskeleton (6). To test whether talin may serve a similar role during Fc receptor-mediated phagocytosis, we studied its distribution in macrophages ingesting IgG-coated particles. We report here that talin colocalizes with F-actin in the submembranous area beneath forming phagosomes in macrophages undergoing Fc receptor-mediated phagocytosis.

Materials and Methods

Cells and Reagents. J774 cells were maintained in spinner culture in DME supplemented with 10% bovine serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Thioglycollate-elicited mouse peritoneal macrophages were harvested 4 d after the intraperitoneal injection of 1 ml of thioglycollate broth (Difco Laboratories, Detroit, MI). Bodipy-phalloidin was from Molecular Probes (Eugene, OR); fluorescein-conjugated goat anti-rabbit IgG and Texas red-conjugated F(ab')₂ goat anti-rabbit IgG was from Jackson Immunoresearch (West Grove, PA); alkaline phosphatase-conjugated goat anti-rabbit IgG was from Promega Biotec (Madison, WI); and anti-sheep IgG was from Diamedix (Miami, FL).

Immunoblotting. 1.3×10^6 J774 cells were suspended in HEPES-buffered saline in the presence of 1 mM EDTA, 0.5 mM PMSF, and 5 mM diisopropylfluorophosphate. Sample buffer (8) was added, the mixture was boiled, the cell extract was subjected to SDS-PAGE using a 7.5% polyacrylamide gel, and the gels were blotted onto nitrocellulose and incubated in saline containing 150 mM NaCl, 10% goat serum, 0.25% gelatin, 0.2% NP-40, 20 mM Tris-HCl, pH 7.2 (TBS), and further incubated with rabbit anti-talin antiserum (1:2,000 in the above buffer), washed in TBS, incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (1:2,500 in TBS), and developed with nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Phagocytosis. For microinjection studies SRBC were opsonized with rabbit anti-SRBC IgG. For conventional immunofluorescence studies, SRBC were opsonized using U188, a mouse mAb of the IgG2b subclass directed against SRBC, kindly provided by Betty Diamond (Albert Einstein College of Medicine, New York). Phago-

cytosis was synchronized by pre-incubating a monolayer of macrophages with opsonized SRBC (IgG-SRBC) in saline containing 125 mM NaCl, 5 mM KCl, 10 mM NaHCO₃, 1 mM KH₂PO₄, 10 mM glucose, 1 mM CaCl₂, 1 mM MgCl₂, 20 mM Hepes pH 7.45 (HBS) at 0°C for 10 min. The monolayer was washed in HBS three times at 0°C, and incubated at 37°C for the times indicated. Cells were fixed in 3.7% formaldehyde in HBS for 10 min and processed for immunofluorescence.

Immunofluorescence. The cells were permeabilized in 0.2% Triton X-100 in TBS for 8 min, washed in TBS, and incubated with either rabbit antiserum directed against mammalian talin or serum from an unimmunized rabbit at a 1:100 dilution for 30 min, washed in TBS and incubated with Texas red-F(ab')₂ goat anti-rabbit IgG diluted 1:50 in TBS for 30 min. The cells were then counterstained with bodipy-phalloidin, diluted 1:10 in TBS for 15 min at 37°C, mounted, and observed by phase contrast and fluorescence microscopy (Photomicroscope III; Carl Zeiss, Inc., Thornwood, NY). The cells were photographed with Tri-X developed at 800 ASA. Control serum showed virtually no staining.

Microinjection of a 190-kD Talin Fragment. Adherent J774 cells were microinjected with an iodoacetamidotetramethylrhodamine-derivatized 190-kD talin fragment (IATR-talin) (9, 10) as described by Graessmann and Graessmann (11), allowed to recover for 4 h, and incubated with IgG-SRBC as described above. The cells were fixed in 3.7% formaldehyde in PBS, permeabilized as described above, and stained with bodipy-phalloidin diluted 1:10 in PBS for 15 min at 37°C. The cells were photographed as described above.

Results and Discussion

We determined that talin is present in J774 cells by Western blot analysis of an extract of these cells. The antiserum was prepared by immunization of rabbits with human platelet talin (12). This antiserum stained a 225-kD protein (Fig. 1), which is the expected size of talin based on the apparent molecular mass of talin from other mammalian cells (6). The additional bands of slightly lower molecular mass probably represent proteolytic fragments of the native molecule. Serum from an unimmunized rabbit did not stain the 225-kD protein or the lower molecular weight fragments (not shown).

At 0°C, the IgG-SRBC attached to the plasma membranes of J774 cells but were not phagocytosed (13), and did not

stimulate the accumulation of F-actin (not shown) or talin (Fig. 2, *arrowheads*) in the cytoplasm adjacent to these particles. In contrast, 1.5 min after warming the J774 cells to 37°C, both F-actin and talin were concentrated beneath forming phagosomes (Fig. 3). Although talin also was identified elsewhere in the cytoplasm, the greater intensity of staining beneath membranes of forming phagosomes suggests that talin was concentrated there. There was excellent correspondence between the cytoplasmic location of F-actin and of talin. Thus, one of the early events in phagosome formation (Fig. 3) involves the recruitment of talin and F-actin to the cortical cytoplasm beneath the nascent phagosome. At a slightly later stage in particle engulfment (5 min), both F-actin and talin were still apparent beneath incompletely engulfed IgG-SRBC (Fig. 4, *arrowhead*). Talin and F-actin largely disappeared from the portions of cytoplasm adjacent to IgG-SRBC that were completely (Fig. 4, *small arrowhead*) or nearly completely (Fig. 4, *arrow*) ingested. To confirm the effect of IgG-SRBC engulfment on talin localization, we microinjected J774 cells with a fluorescently labeled 190-kD fragment of talin (IATR-talin). Previous work showed that this talin fragment, like native talin, localizes to focal adhesion sites in fibroblasts (10). In unstimulated J774 cells, the 190-kD talin fragment was distributed in the cytosol and in membrane ruffles (not shown). In J774 cells ingesting IgG-SRBC, the 190-kD fragment of talin was concentrated in portions of the cortical cytoplasm directly beneath forming phagosomes, in pseudopods advancing around the particles (Fig. 5, *arrowheads*), and in membrane ruffles. Analogous to conventional immunofluorescence (Figs. 2-4), there was excellent correspondence between the distribution of IATR-talin and F-actin in the cortical cytoplasm of J774 cells engulfing IgG-SRBC.

We obtained similar results in thio-macrophages. 1.5 min after the onset of ingestion at 37°C, talin (Fig. 5) and F-actin (not shown) localized beneath forming phagosomes. At later time points, neither talin nor F-actin were apparent adjacent to fully ingested particles.

The recruitment of talin during Fc receptor-mediated phagocytosis is surprising in two respects. First, although previous studies have documented the presence of talin at sites

200-
93-
69-
43-



Figure 1. Immunoblot of J774 cell extract stained with rabbit antitalin antiserum. Positions of molecular mass markers in kilodaltons are listed.

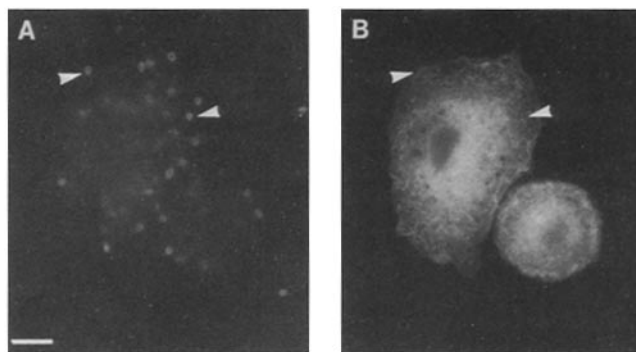


Figure 2. Immunofluorescent staining of IgG-RBC and talin in J774 cells incubated with IgG-RBC for 10 min at 0°C. Note the absence of talin localization adjacent to IgG-RBC (*arrowheads*). (A) Fluorescein-conjugated anti-rabbit IgG (which stains IgG-RBC); (B) talin immunofluorescence. Bar = 10 μ m.

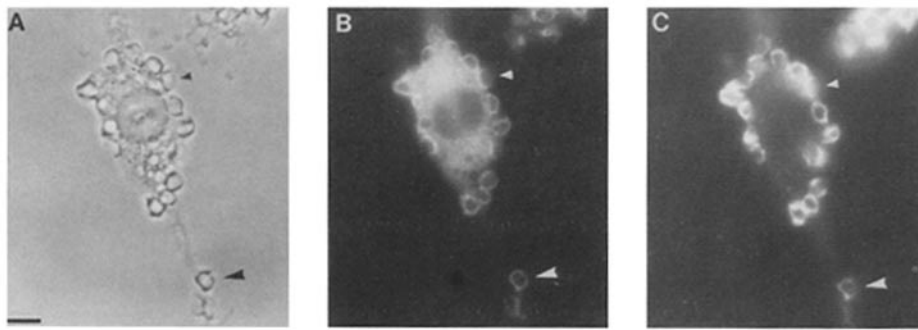


Figure 3. Fluorescent staining of F-actin and talin in J774 cells undergoing phagocytosis for 1.5 min. Note the colocalization of F-actin and talin staining adjacent to nascent phagosomes (*small arrowhead*). Although some cytosolic staining is evident, one phagosome (*large arrowhead*) shows a concentration of talin, while the surrounding cytosol has almost no detectable talin. (A) Phase contrast; (B) talin immunofluorescence; (C) F-actin fluorescence. Bar = 10 μ m.

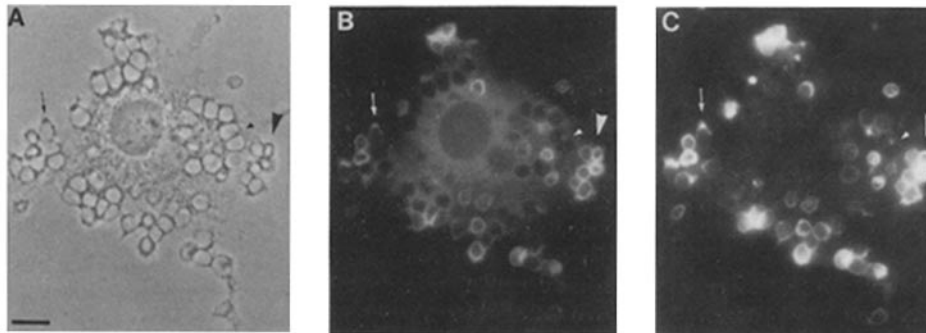


Figure 4. Fluorescent staining of talin and F-actin in J774 cells undergoing phagocytosis for 5 min. Note that talin and F-actin are not visible surrounding particles that have been completely engulfed (*small arrowhead*), but are associated with particles that are being engulfed (*large arrowhead*). F-actin and talin are visible only at the distal tip of a pseudopod encircling one particle that is nearly completely ingested (*arrow*). (A) Phase contrast; (B) talin immunofluorescence; (C) F-actin fluorescence. Bar = 10 μ m.

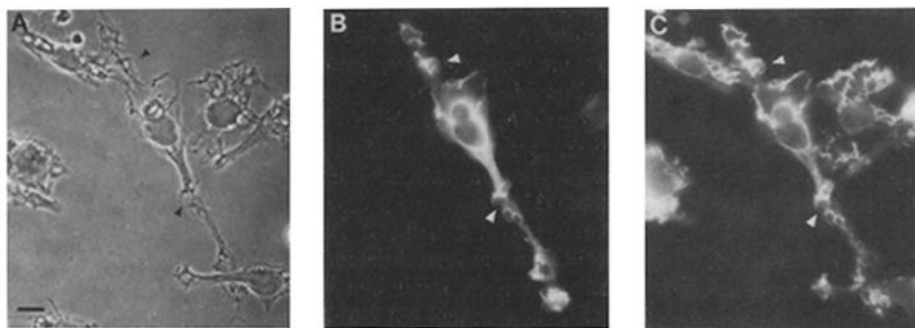


Figure 5. Localization of F-actin and microinjected IATR-talin in J774 cells undergoing phagocytosis for 1.5 min. Note the colocalization of F-actin and the fluorescent talin fragment in pseudopods (*arrowheads*) and membrane ruffles. (A) Phase contrast; (B) fluorescence of IATR-talin; (C) F-actin fluorescence. Bar = 10 μ m.

of contact of mononuclear cells with their substrates, the talin appeared at these sites only after many hours of adherence (14). In contrast, particle engulfment is a transient and dynamic process. Second, talin binds to the cytoplasmic domains of integrins (15), at sites where stress fibers insert (6). In con-

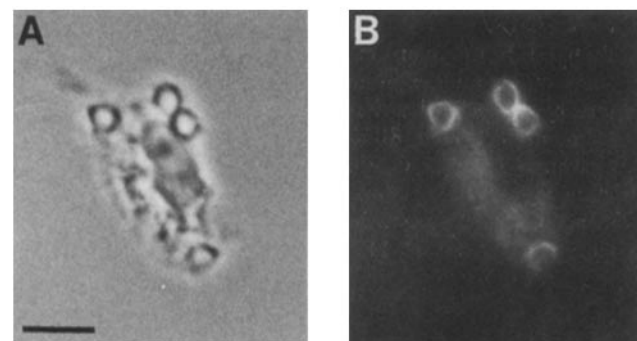


Figure 6. Fluorescent staining of talin beneath forming phagosomes in thio-macrophages undergoing phagocytosis for 1.5 min. (A) Phase contrast; (B) talin immunofluorescence. Bar = 10 μ m.

trast, macrophages exhibit few, if any, stress fibers. The presence of talin in the cortical cytoplasm adjacent to forming phagosomes suggests that it may have a role in connecting the microfilament-based cytoskeleton to the plasma membrane during phagocytosis.

Talin redistribution has been demonstrated in helper and cytotoxic T cells during contact with their respective targets (16, 17), as well as during activation of platelets by thrombin (18). Talin also has been reported to localize to the cortex of transformed fibroblasts as they engulf fibronectin-coated beads (19). These results are not totally unexpected, since in each of the instances cited integrins play a central role. However, Fc receptors bear no sequence homology to the known integrins (20). Whether integrins participate as cofactors in Fc receptor-mediated phagocytosis, as has been suggested (21–23), remains to be clarified. In any case, the identification of talin in the cortex of macrophages during Fc receptor-mediated phagocytosis suggests a new function for talin, and raises the possibility that other talin-associated proteins (e.g., α -actinin) participate in the engulfment process as well.

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