Phagocytosis is initiated by the engagement of phagocyte surface receptors by a particulate target, which triggers the recruitment and activation of a variety of signaling molecules, culminating in the local reorganisation of the actin cytoskeleton and internalisation of the bound particle. The most extensively characterised phagocytic pathway to date stems from the Fcγ receptor. Ligation of the FcγR by an IgG-opsonised particle stimulates pseudopodial extensions of the cell membrane that surround and eventually ensnare the particle. It is firmly established that dynamic actin polymerisation is essential for this process, however, it is less well understood how the localised contractile activity which constrains the margins of forming phagosomes coordinates with the actin cytoskeleton to extend pseudopods and close the phagocyte cup (Swanson et al., 1999). Several different motor proteins have been implicated in mammalian phagocytosis including members of the class I myosins, myosin IIA, myosin X and myosin Va (reviewed in Araki et al., 2006).

Myosins bind actin filaments and generate mechanical force by hydrolysing ATP. In general, their structure consists of an N-terminal head or motor domain encompassing the ATP- and actin-binding sites; a neck region containing pseudopodial extensions of the cell membrane that surround and eventually ensnare the particle. It is firmly established that dynamic actin polymerisation is essential for this process, however, it is less well understood how the localised contractile activity which constrains the margins of forming phagosomes coordinates with the actin cytoskeleton to extend pseudopods and close the phagocyte cup (Swanson et al., 1999). Several different motor proteins have been implicated in mammalian phagocytosis including members of the class I myosins, myosin IIA, myosin X and myosin Va (reviewed in Araki et al., 2006).

Herein we propose that the class I myosin, myosin IG is recruited to phagocytic cups upon ligation of the FcγR. Our data suggest that its localisation at the phagocytic cup is controlled by the activity of PI3K and that two conserved basic residues present in a PH-like domain of the tail are necessary for the functioning of myosin IG in internalisation of IgG-coated particles.

**Summary**

Myosin IG localisation at phagocytic cups. Their localisation coincides with the appearance of F-actin, peaking in the presence of the inhibitor. Furthermore, both the motor domain and the PH-like domain of myosin IG are important for engulfment downstream of the FcγR.

**Figure 1:** J774A.1 macrophages were pre-incubated with 5 μM of the PI3K inhibitor ML-7 or the DMSO vehicle and then incubated with IgG-opsonised beads for 15 minutes at 37 °C. Representative images of GFP-Myo1G co-localising with F-actin in J774A.1 macrophages were captured in the presence of the inhibitor. % phagocytosis, defined as the proportion of total bound IgG-beads, was assessed by differential interference contrast florescence pre- and post-pseudopod formation. Results are the mean ± S.E.M of triplicate experiments. *p<0.05, **p<0.01. a) Characteristic images of GFP-Myo1G co-localising with F-actin in a phagocytic cup. Scale bar, 10 μm. b) Representative examples of GFP-Myo1G localisation in FcγR-expressing COS-7 cells with enlargement of sites of particle binding shown. Scale bar, 10 μm. c) Recruitment of GFP fusion protein to phagocytic cups over time was scored. Mean ± S.E.M of 3 experiments, each with n=20 transfected cells per experiment. *p<0.05. **p<0.001.

**Figure 2:** a) Schematic of the domain structure of the class I myosin, myosin IE. b) COS-7 cells were co-transfected with FcγR and GFP-myosin IE and challenged with IgG-opsonised beads for either 5, 10 or 15 minutes at 37°C. Representative example of GFP-MyoIE localisation with enrichment at sites of particle binding. Scale bar, 10 μm. c) Recruitment of GFP fusion protein to phagocytic cups over time was quantified. d) Schematic of the domain structure of myosin IG. e) J774A.1 macrophages were transfected with IgG-beads for 15 minutes and then labelled with phalloidin to detect F-actin and an anti-Myo1G antibody (Rockland, Inc). Representative image of GFP-Myo1G co-localising with F-actin and WGA to detect membrane. Arrow denotes a bead that is myosin IIA-positive and co-localises with the membrane marker. Scale bar, 10 μm.

**Figure 3:** J774A.1 macrophages were pre-incubated with 50 μM of the PI3K inhibitor LY294002 or the DMSO vehicle and then incubated with IgG-opsonised beads for 15 minutes at 37 °C in the presence of the drug. The recruitment of GFP-Myo1G to phagocytic cups under these conditions was quantified as % positive phagocytic cup. p<0.05. e) Representative confocal images of GFP-Myo1G localisation in FcγR-expressing COS-7 cells that have been incubated in either DMSO or LY294002. Scale bar, 10 μm.

**Figure 4:** a) Schematic representation of the various truncation and point mutants of Myo1G used in this study. b) COS-7 cells were co-transfected with the FcγR and the indicated constructs before being challenged with IgG-beads for 15 minutes at 37°C. Characteristic images of GFP-fusion protein localisation with enlargements of sites of particle binding are shown. Scale bar, 10 μm. c) Phagocytosis was determined as the number of internalised IgG-beads per 100 transfected cells and was expressed as a percentage. Data represent the mean ± S.E.M of 3 independent experiments. p<0.05.

**Working Questions....**

- Is the localisation and requirement for Myo1G at the phagocytic cup dependent on particle size?
- What is the phosphoinositide binding specificity of the PH-like domain?
- Are these myosins involved in CR3-mediated phagocytosis?

**Conclusions**

We have shown that:

- Myosin function is important for FcγR phagocytic cup formation.
- Two unconventional class I myosins, myosin IE and Ig are enriched at FcγR phagocytic cups. Their localisation coincides with the appearance of F-actin, peaking 15 minutes after IgG-bead binding.
- Myosin IG recruitment to the FcγR phagocytic cup is dependent on PI3K activity.
- The motor domain and the conserved basic residues in the PH-like domain of the tail of myosin IG are important for engulfment downstream of the FcγR.

Our results identify the involvement of a novel class I myosin, myosin IG in FcγR-mediated phagocytosis and suggest that this myosin is a downstream target of PI3K activity. Furthermore, both the motor domain and the PH-like domain of myosin IG contribute to the necessity of myosin IG for uptake following FcγR ligation.