Biophysical studies of mechanotransduction associated with CR3 induced phagocytosis

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Phagocytosis is the mechanism of internalization of large particles of several microns in size and therefore, it involves important mechanical constraints. Actin polymerization provides the driving force of membrane deformation that is a necessary feature of phagocytosis: a transient burst of actin polymerization associated with the formation of the phagocytic cup. CR3 induced phagocytosis is less well characterized than other phagocytic pathways. Like other integrins, CR3 needs an 'inside-out' signal to reach an active conformation. Although the biochemical activities of integrin regulators are known, the mechanisms by which these mechanosensitive protein machineries sense force and modulate actin dynamics and anchoring during CR3 induced phagocytosis has not been described. A necessary first step to address this paucity is the determination of the sequential recruitment of signaling proteins (including vinculin, Talin1, FilaminA and B) in the phagocytic cup. Initial experiments with primary human macrophages were performed using widefield deconvolution microscopy. Macrophages were allowed to phagocytose complement opsonized red blood cells and were stained with anti-filamin A, anti-vinculin antibodies and phalloidin to detect F-actin. RAW264.7 macrophages were visualised on complement coated slides that induce phagocytosis but frustrate its progression by total internal reflection fluorescence microscopy (TIRFM). Macrophages were transfected to express Lifeact-mCherry to detect F-actin, which for CR3 induced phagocytosis, was observed to accumulate in patches at the center and edges of the spreading zone contrasting with the ring-like structure formed in FcR induced phagocytosis. Together, these data demonstrate that we can observe the sequential arrival and departure of these proteins with parallel actin assembly and disassembly, constituting a 'proof of concept' for our future studies of CR3 induced phagocytosis, and the proteins and forces that regulate it.