

## A ROLE FOR CYTOSOLIC BRAIN CREATINE KINASE IN PHAGOCYTOSIS.

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Important cellular functions like control of cell shape, migration and phagocytosis depend on rapid reorganization of the actin cytoskeleton, which is driven by actin polymerization and myosin action. Both actin polymerization and myosin action consume ATP and depend on adequate ATP/ADP ratios.

The creatine kinase (CK) system preserves optimal local ATP/ADP ratios and delivers ATP to sites of energy consumption. Therefore, CK could be important in rapid actin-remodeling processes. This also implies that CK could become spatio-temporally located to sites of action. Cytosolic brain-type CK (CK-B) and ubiquitous mitochondrial CK exhibit broad tissue distribution, but are most prominently expressed in brain. CK-B was also the major isoform identified in macrophages (Loike et al., 1984), which prompted us to investigate the role of CK-B in actin-remodeling during phagocytosis.

Macrophage (Raw 264.7) derived cell lines were transiently transfected with CK-B and fixed during phagocytosis of zymosan particles. Indirect immunofluorescence revealed accumulation of both actin and CK-B in the phagocytotic cup. Using GFP-tagged CK-B in living cells we observed that accumulation in the phagocytotic cup is only temporal during several minutes in the initial phase of phagocytosis of the particle.

Since these observations point to spatio-temporal co-accumulation of actin and CK-B, we established cell lines stably co-expressing actin-GFP (Raw 264.7 cells kindly provided by Dr. F. van Leeuwen, Dept. of Tumor Immunology, NCMLS) and CKB-CFP to analyze this further. We demonstrate that endogenous CK-B in macrophages is mobilized from the cytosolic pool and coaccumulates with F-actin at nascent phagosomes. Live cell imaging revealed the transient and specific nature of this partitioning process. Overexpression of a catalytic dead CK-B or CK-specific cyclocreatine inhibition caused a significant reduction of actin accumulation in the phagocytic cup area, and reduced complement receptor-mediated, but not Fc- $\gamma$ R-mediated, ingestion capacity of macrophages. Finally, we found that inhibition of CK-B affected phagocytosis already at the stage of particle adhesion, most likely via effects on actin polymerization behavior. We propose that CK-B activity in macrophages contributes to complement-induced F-actin assembly events in early phagocytosis by providing local ATP supply.

J.W.P. Kuiper, H. Pluk, F. Oerlemans, F.N. van Leeuwen, F. de Lange, J. Fransen and B. Wieringa, "Creatine kinase-mediated ATP supply fuels actin-based events in phagocytosis." *PLoS Biol* **6**, e51 (2008).