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A role for calcineurin in Dictyostelium discoideum phagocytosis

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The Ca²⁺/calmodulin-dependent protein phosphatase calcineurin is involved in the development of the cellular slime mold *Dictyostelium discoideum*. Because of its interactions with Ca²⁺, which appear to influence *D. discoideum* phagocytosis (Yuan and Chia, 1999, *Mol. Biol. Cell* 10, 220a), we undertook studies to test whether calcineurin also plays a role in *Dictyostelium* phagocytosis. The immunosuppressants cyclosporin A and FK506, through the formation of cyclosporin A-cyclophilin A and FK506-FK506-binding protein complexes, respectively, inhibited calcineurin activity. These two calcineurin inhibitors suppressed phagocytosis of fluorescently labeled yeast in a dose-dependent manner. Although it inhibited phagocytosis, cyclosporin A had an insignificant effect on the macropinocytosis of the fluid-phase marker fluorescein isothiocyanate-dextran. Furthermore, trifluoperazine, a calmodulin antagonist that indirectly inhibits calcineurin, also suppressed phagocytosis in a dose-dependent fashion and induced the formation of giant intracellular vacuoles. Fluorescence microscopy of cyclosporin A-treated (for 30 min.) cells stained with rhodamine-phalloidin had cytoplasmic chunks of F-actin that were not present in control cells, while cells treated with FK506 and trifluoperazine (also for 30 min.), displayed less cortical but more cytoplasmic F-actin staining than normal cells. Typically, drug-treated cells were smaller and rounder than untreated cells. Our data suggest calcineurin may play a role in *D. discoideum* phagocytosis, either through the dephosphorylation of actin-regulating proteins or other cytoskeletal proteins such as the heavy chain subunit of nonmuscle myosin II since dephosphorylation of the latter promotes filament assembly.