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Modulation of macrophage function by the proteins secreted from the RD-1 region of *M. tuberculosis*

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Mycobacterium tuberculosis (Mtb) is perhaps the most successful intracellular pathogen known to persist for long periods inside the macrophage. The proteins secreted by Mtb have gained an increased attention as potential virulence factors or vaccine candidates. Some of them, *e.g.,* CFP-10 (MTSA-10) and ESAT-6 encoded by the RD-1 region of the Mtb genome are missing in all the BCG strains of *M. bovis*, and are therefore attractive targets for such studies. First we have delineated possible role of MTSA-10 in modulation of the host macrophage functions. Using two-dimensional gel electrophoresis technology, we found that recombinant MTSA-10 induced murine macrophage cells (J774.1) to undergo extensive de-phosphorylation of the total phospho-proteome. About two-thirds of the proteins got de-phosphorylated within 20 minutes of treatment. This dephosphorylated state persisted without any significant change for 1 hr, and might be the key event that contributes to macrophage dysfunction. Macrophages produce generous amounts of reactive oxygen species (ROS) after an encounter with antigens. However, MTSA-10 caused a reduction in ROS production by J774.1 cells. Furthermore, we found that MTSA-10 stimulation activated a whole array of protein tyrosine and Ser/Thr phosphatases, which bring about extensive de-phosphorylation of macrophage proteins.

ROS is known to inactivate phosphatases and thereby activate the kinases. MTSA-10 appeared to keep the phosphatases in active state. This implies that MTSA-10 could activate phosphatases by lowering down the ROS production in macrophages. Our findings suggest that MTSA-10 might impart *Mycobacterium* with an ability to not only evade destruction but also survive within the macrophage.

We also show that ESAT-6, a leading vaccine candidate, is involved in modulation of the mitogen-activated protein (MAP) kinase signaling pathway inside the macrophage. ESAT-6 binds to the surface of RAW264.7 cells and induces phosphorylation of the extracellular signal regulated kinase ½ (ERK 1/2) in the cytoplasm of RAW264.7 cells. It is known that MAP kinases after being phosphorylated translocate into nucleus where they phosphorylate and activate downstream transcription factors; however, in the present study, no phospho-ERK1/2 could be detected in the nucleus, instead, we observed that unphosphorylated ERK1/2 accumulated in the nucleus, indicating active dephosphorylation of the translocated phospho-ERK1/2. Treatment of cells with sodium vanadate (tyrosine phosphatase inhibitor) followed by stimulation with ESAT-6 caused reappearance of phospho-ERK1/2 in the nucleus, thus indicating the involvement of some putative phosphatase(s) in this phenomenon. Furthermore, ESAT-6 downregulated LPS-induced *c-myc* expression via inhibiting ROS production, and NF-κB transactivation. This could dampen the overall activation response of the macrophage. The study reveals a novel possible function of ESAT-6 in modulating the host cell responses and contributing to mycobacterial pathogenesis.