INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	**SOORAM BANESH**
Roll Number	:	**156106009**
Programme of Study	:	**Ph.D.**

Thesis Title: **Potentials of CD36 in sensing apoptotic cells and modulating hemin mediated immune response from macrophages.**

Name of Thesis Supervisor(s)	:	**Prof. Vishal Trivedi** and **Prof. Vibin Ramakrishnan**
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SHORT ABSTRACT

The macrophages express various receptors on their surface to assist in the innate immune response. The scavenger receptors expressed on the macrophage cell surface are involved in a broad range of functions, including metabolism, sensing of biomolecules, and regulating homeostasis in the host. Among the scavenger receptors, the class B receptor CD36 is of interest to many researchers because of its indispensable functional roles in angiogenesis, inflammation, and pathogen clearance from the body. The CD36 has been shown to interact with PS expressing apoptotic cells, but the molecular features that enable the binding of CD36 to PS/apoptotic cells are elusive. To investigate the binding of PS to CD36, we have cloned, overexpressed, and purified CD36 ectodomain in the bacterial expression system. The dot-blot analysis and isothermal titration calorimetry (ITC) show that the hCD36_ecto selectively binds to the PS with a dissociation constant (KD) of 53.7 ± 0.48 μM. The molecular modeling study indicates that the residues R63, N118, and D270 are crucial for binding PS to CD36. The dot blot assay and ITC based affinity studies further confirm the R63 and D270 are essential for PS binding. These findings may also explain the molecular basis of binding of CD36 to apoptotic cells.

Further, the hemoglobin derivative products (hemin) released during RBC lysis may serve as potential ligands for macrophage surface receptors. During cerebral malaria, hemin levels elevated and correlating with TNF-α and MCP-1 cytokines; however, the molecular mechanism behind it is not well understood. Macrophages treated with hemin exhibited immune dysfunction and CD36 translocation into intracellular

storage. The molecular modeling study has suggested that the CD36 has a well-defined region to accommodate hemin and consists of R292, D372, and Q382. The affinity studies have indicated that the wild type (hCD36ecto) interacts with hemin with high affinity, and the mutation in biophore residues (R292A, D372A, or Q382A) significantly reduced the affinity. The migration assay in MG63 cells (low levels of CD36 and TLRs) with CD36 ectopic expression has confirmed that the membrane-bound CD36 with intact biophore is essential for hemin interaction. Further, the MG63 cells with wild type CD36 expression showed several folds increment in cytokines TNF-α, MCP-1, RANTES, and CCL1 in response to hemin stimulation, but no significant amount of cytokines released in mutants (R292A, D372A, or Q382A) validates the hemin act as a ligand for CD36 and responsible for immune-dysfunction. The phosphoprotein western blot and immunoprecipitation studies have revealed that hemin activating the downstream signaling through phosphorylation of CD36 and subsequent recruitment of Src family kinase protein to the cytosolic domain of CD36. The Lyn targeted siRNA restored the phagocytic activity, reduced the pro-inflammatory cytokine levels to normal, and suggested that the Src family protein Lyn is crucial for cytokine signaling. In summary, the hemin-CD36-Lyn cytokine signaling axis could be a contributing factor to severe malaria pathology and prognosis. To identify hemin-CD36 interaction blockers, several compounds from the phytochemical and FDA-approved drug banks screened against the hemin binding site. Gallic acid from the phytochemical pool and Meropenem from the FDA-approved drug bank was effective in selectively reducing hemin-CD36 mediated immune-dysfunction. In summary, the study findings provide insights into molecular features that enable CD36 interaction with PS and hemin and suitable adjuvant molecules that ameliorate hemin-mediated immune dysfunction in macrophages.

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