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Characterization of Proteins derived from *Wolbachia* of *Brugia malayi* associated with Proinflammatory Responses

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Abstract

Objective: *Wolbachia* are obligate intracellular bacteria found widespread in arthropods and filarial nematodes. Besides their role in worm's reproduction and development, *Wolbachia* are associated with immunopathogenesis of filariasis. The objective of this study is to characterize proteins derived from *Wolbachia* of *Brugia malayi* and their proinflammatory activity on macrophage cell line.

Methods: Proteins *of Wolbachia*–enriched extracts from B. *malayi* were separated by high–resolution two–dimensional gel electrophoresis. Spots specific for *Wolbachia* were detected by anti–*Wolbachia* antibodies. A gene–expressing candidate *Wolbachia* protein was cloned and induced in *E. coli* to express as a recombinant protein. The murine macrophage cell line RAW 264.7 was incubated with the recombinant protein, and expression of proinflammatory cytokines mRNA in RAW 264.7 cells was evaluated by real–time reverse transcriptase–polymerase chain reaction (real–time RT–PCR).

Results: The reactive spots specific for *Wolbachia* of *B. malayi* were detected and compared to spots detected on the Coomassie blue–stained 2D gel. We could identify a major protein as *Wolbachia* surface protein, WSP. *wsp* gene was subsequently cloned and induced to express as recombinant WSP. Interleukin–1β (IL–1β), tumor necrosis factor alpha (TNF–α), and interleukin–6 (IL–6) mRNA in the murine macrophages were up–regulated by rWSP stimulation in a dose–dependent manner. The induction of all cytokines was abolished by Proteinase K treatment of the rWSP.

Conclusion: *Wolbachia* surface protein (WSP) is a major spot showing strong antigenicity. In addition, WSP represents as an inflammatory molecule that is capable of directly stimulating the macrophages to produced cytokines.