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Paper Poster Session

Non-tuberculous mycobacteria

Mycobacterium marinum mmar_2318 and mmar_2319 are responsible for lipooligosaccharide biosynthesis and virulence towards Dictyostelium

Yi-Yin Chen^{*1}, Feng-Ling Yang², Shih-Hsiung Wu², Tzu-Lung Lin¹, Jin-Town Wang¹

¹National Taiwan University College of Medicine, Department of Microbiology, Taipei, Taiwan

²Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Background: Mycobacteria possess a unique lipid-rich cell wall that is important in directing host-pathogen interactions and confers resistance to many therapeutic agents. *Mycobacterium marinum* can cause a systemic tuberculosis-like infection in fish, ectotherms and human, a process that involves persistent growth within macrophages. *Dictyostelium*, a free-living amoeba, serves as a macrophage-like system for studying bacteria-host interactions. A well-established model system using *Dictyostelium discoideum* was introduced for studying the interactions between phagocytes and bacteria.

Material/methods: We constructed a *M. marinum* mutant library (1728 transposon mutants of the NTUH-M6094 strain) by transposon mutagenesis and used a *Dictyostelium* phagocytosis plaque screening model to identify genetic loci involved in *M. marinum* virulence. The unmarked deletion and complementation of target genes were generated. Then their virulence to *Dictyostelium*, colony morphology, glycolipid profile, as well as entry/replication inside *Dictyostelium* and mammalian macrophages (J774a.1 and THP-1) were analyzed.

Results: The screening identified a total of 30 mutants permissive for *Dictyostelium* growth. These mutants revealed interruptions in 20 distinct loci. Of the 20 loci, six genes (*losA*, *mmar_2318*, *mmar_2319*, *wecE*, *mmar_2323* and *mmar_2353*) were located in the lipooligosaccharide (LOS) synthesis cluster. LOS are antigenic glycolipids and the core LOS structure from LOS-I to LOS-IV have been reported to exist in *M. marinum*. Two-dimensional thin-layer chromatography (2D-TLC) glycolipid profiles revealed that deletion of *mmar_2318* or *mmar_2319* resulted in the accumulation of LOS-III and deficiency of LOS-IV. The *mmar_2318* or *mmar_2319* deletion mutants also exhibited rough colony morphology and bigger colony size in comparison with wild type. Deletion and complementation of *mmar_2318* or *mmar_2319* confirmed that these genes both contributed to virulence towards *Dictyostelium* but not entry and replication inside *Dictyostelium*. Co-incubation with a murine macrophage cell line J774a.1 or PMA-induced human monocytic cell line THP-1 demonstrated that *mmar_2318* or *mmar_2319* deletion mutant could grow in macrophages, and their initial entry rate was not affected in J774a.1 but significantly increased in THP-1.

Conclusions: Although *mmar_2319* has been reported to involve LOS biosynthesis in a previous study, we identified a new gene, *mmar_2318* that is also involved in the biosynthesis of LOS. Deletion of *mmar_2318* or *mmar_2319* both exhibits reduction of virulence towards *Dictyostelium*, and increased entry into THP-1 cells.