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Recombinant *Mycobacterium leprae* protein associated with entry into mammalian cells of respiratory and skin components

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Endothelial cells;
Leprosy

Summary

Backgrounds: The transmission of *Mycobacterium leprae*, the causative pathogen of leprosy, has been postulated to occur mainly through upper respiratory route rather than skin-to-skin contact via minor injuries. The *M. leprae* genome contains *mce1A* gene, which encodes a putative mammalian cell entry protein. However, to date, there have been no functional analyses of the *M. leprae mce1A* gene product.
Objective: The aim of this study was to elucidate a possible relationship between this transmission mechanism and the *mce1A* gene product.
Methods: We analyzed the cell uptake activity *in vitro* of polystyrene latex beads coated with a purified recombinant (r-) protein expressed by a 849-bp locus within the *mce1A* gene.
Results: The r-protein promoted uptake of the beads into human nasal epithelial cells derived from nasal polyps, human bronchial epithelial cell line, normal human dermal fibroblasts, normal human microvascular endothelial cells and normal human keratinocytes cultured at 0.01 mM extracellular calcium concentration [Ca]; no uptake occurred with keratinocytes cultured at 1.2 mM [Ca].
Conclusion: These results suggest that the *mce1A* gene product can mediate *M. leprae* entry into respiratory epithelial cells as their natural target cells, which may be the main mode of transmission. Endothelial cells, on the other hand, may serve as the reservoir of the bacilli for long-term infection. The *M. leprae* Mce1A protein has

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potential important implications for mode of transmission and pathogenesis of leprosy.

1. Introduction

Leprosy is a chronic granulomatous infection of the skin and peripheral nerves with the intracellular bacterium *Mycobacterium leprae*, and a great deal of research has been conducted on its transmission mode. Two routes — through the nasal mucosa and through minor skin injuries [1] — have been postulated as the principal modes of transmission of *M. leprae*, but the nasal mucosa has been recently suggested to be the principal route [2]. The mechanism of entry into the nasal mucosa or skin by *M. leprae*, however, has not been clearly elucidated.

The *mce1A* gene in *mce1* operon (mammalian cell entry) of *Mycobacterium tuberculosis* genome is a virulence gene involved in epithelial cell entry and survival inside macrophages [3]. The recombinant (r-) *M. tuberculosis* Mce1A protein has been shown to promote epithelial cell uptake of latex microspheres coated with the r-protein [4], and the ability of epithelial cell entry can be conferred upon non-pathogenic *Escherichia coli* by expressing it on their surface [5]. The *M. leprae* genome sequence [6] has revealed a region containing eight genes (*yrbE1A*, *yrbE1B*, *mce1A*, *mce1B*, *mce1C*, *lprK*, *mce1E* and *mce1F*) with close identity of the encoded proteins to the *M. tuberculosis mce1* operon. The protein encoded by *M. leprae mce1A* gene is a putative epithelial cell entry protein, suggesting that it participates in the entry of *M. leprae* into the nasal mucosa and skin. However, to date, there have been no functional analyses of the *M. leprae mce1A* gene product.

To elucidate the role of *M. leprae mce1A* gene in the transmission mode of leprosy, we generated a r-protein and investigated its entry activity in respiratory epithelial cells as well as the cells of skin components, which comprise the possible initial transmission routes of *M. leprae*.

2. Materials and methods

2.1. Bacterial strain and plasmid

The genomic DNA used in the study was isolated from *M. leprae* strain Thai 53, which was maintained at Leprosy Research Centre, National Institute of Infectious Diseases, Japan, as previously described [7]. The pQE30 plasmid and *E. coli* M15 [pREP4] were purchased from Qiagen (Valencia, CA, USA). The pQE30 plasmid was used as expression vector. *E. coli* M1 5 [pREP4] was used as a host for the vector, as recommended by the manufacturer. The use of pQE30 vector allowed the expression of Mce1A protein of *M. leprae* with a polyhistidine (6 × His) tag at the N-terminus (r-Mce1a).

2.2. Construction of vector

In Sanger Centre *M. leprae* strain TN complete genome sequence, *mce1A* gene is a 1326-bp putative open reading frame (ORF) located between positions 3092446 and 3093771 (NCBI-GeneID: 910890). The *mce1A* DNA sequence of strain Thai 53 was identical to that of strain TN. It was subcloned into pQE30 vector in a truncated reading frame. The 849-bp ORF deleted at 5′ and 3′ ends of *mce1A* gene is located between positions 73 and 921 (Fig. 1A). This sequence was amplified by polymerase chain reaction (PCR) directly from the genomic DNA of *M. leprae* strain Thai 53 with oligonucleotide primers designed

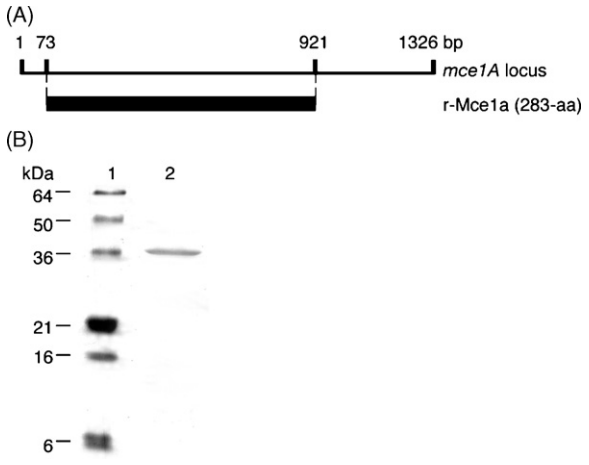

Fig. 1 DNA sequence and purification of the recombinant *M. leprae* truncated Mce1A protein (r-Mce1a). (A) The full-length Mce1A protein is encoded by 1326-bp ORF indicated as *mce1A* locus in the *M. leprae* strain TN genome sequence. The r-Mce1a represents a truncated protein with 283-amino-acid residues deleted at the N- and C-terminus of the full-length Mce1A protein. (B) Coomasie brilliant blue stain of a 12% SDS-PAGE gel reveals a purified 37-kDa protein (lane 2) expressed by 849-bp ORF DNA fragment cloned from *M. leprae* strain Thai 53. Lane 2 contains about 2 μg of a protein. Lane 1 contains a molecular weight marker.