Mol Cell Biochem (2014) 385:43–52
DOI 10.1007/s11010-013-1813-5

Prokaryotic expression and functional analysis of the *Mb1514* gene in *Mycobacterium bovis*

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Received: 30 March 2013 / Accepted: 13 September 2013 / Published online: 20 October 2013

Abstract The ability of mycobacteria to grow and invade target tissues is the key component in the process of *Mycobacterium bovis* infection. Therefore, analysis of the proteins responsible for cell invasion will assist clinicians in combating bovine tuberculosis. The *Mb1514* gene of *M. bovis* encodes a hypothetical invasion protein (designated here as MbINV protein), whose function has not yet been directly identified. In this study, the *Mb1514* gene from *M. bovis* was cloned, and expressed in *E. coli*. The recombinant MbINV protein (a single band of approximately 28 kDa) was purified for biological analysis. Our data demonstrated that recombinant MbINV protein significantly inhibited the viability of RAW264.7 macrophages in a dose-dependent manner ($P < 0.05$), and induced cell necrosis, indicating that the protein is toxic. MbINV protein infection significantly enhanced the mRNA expression levels of TNF-α, IL-1β, and NOS2 ($P < 0.01$), suggesting that MbINV protein may be one of the virulence factors which directly interact with macrophages and modulate the host immune response to *M. bovis*. An invasion inhibition assay showed that MbINV-inhibited *M. bovis* invasion of RAW264.7 cells in a concentration-dependant manner, demonstrating it is an invasion protein.

Keywords *Mycobacterium bovis* · Invasion protein · *Mb1514* · RAW264.7 · Proinflammatory cytokines

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Introduction

Bovine tuberculosis (bTB) is an infectious disease of cattle caused by *Mycobacterium bovis* (*M. bovis*), a member of the Mycobacterium tuberculosis (MTB) complex, classified as a level 3 pathogen for public health (OIE, 2005). The cost of bTB to the global agricultural industry is an estimated $3 billion annually [1]. *M. bovis* has a wide host range [2] and spillover of infection from various species to livestock or other wildlife hosts causes difficulties in eradication [3, 4]. Potential bTB infection of humans through inhalation, ingestion, or less frequently, through absorption by contact with mucous membranes and broken skin, is a cause of significant public health concern. In the developing world, *M. bovis* may account for up to 10 % of cases of human TB, especially in the context of coinfection with HIV/AIDS [5]. Thus, the increased incidence of bTB, aside from being a major economic problem, poses an increasing risk to human health, and is recognized as a global threat at the human–livestock–wildlife interface and a clear "One Health" issue [6]. Therefore, there is an urgent need to develop new approaches to combat bTB. In the struggle to develop more effective vaccines and therapeutics to control this debilitating disease, investigators require an improved understanding of the basic molecular physiology of the underlying pathogen involved.

The ability of mycobacterium to grow and invade is the key component in the etiology of *M. bovis* infection. The *Mb1514* gene of *M. bovis* (AF2122/97) encodes a hypothetical invasion protein [1]. A series of studies have been performed on several homologs of this gene, including *Rv1478* in *Mycobacterium tuberculosis* from the H37Rv strain (100 % identity), *MAP1204* in *M. avium* subsp. *Paratuberculosis* (77.82 % identity) and mycobacterial invasion and intracellular persistence factor (iipB) in