Mycobacterium avium

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In this study we characterized adhesins heparin-binding hemagglutinin (HBHA) and laminin-binding proteins (LBP) from *M. intracellulare* subsp *chimaera intracellulare* complex (MCIC) species isolated from patients with a variety of disease expression, examined the role of these adhesins in binding of *M. intracellulare* to lung epithelial cells and their degree of conservation within the *M. intracellulare* subsp *chimaera intracellulare* complex

Keywords: Mycobacterium intracellulare ; NTM ; adhesins

1. Introduction

Non-tuberculous mycobacteria (NTM) are an increasing cause of opportunistic diseases in humans [1][2]. Among NTM, the *Mycobacterium avium* complex (MAC) represents a group with a specific distribution of species according to continent and countries [3]. In addition to severe infection in immune-deficient subjects, such as AIDS patients, the incidence of MAC infections has also recently increased in patients with chronic pulmonary disease and other underlying conditions [4][5]. Due to modifications in mandatory programs of vaccination with bacillus Calmette-Guérin (BCG) in low-incidence countries, an increase in the frequency of adenitis in children was noticed [6] mostly because of MAC infection. MAC is classically divided into *Mycobacterium avium* and *Mycobacterium intracellulare*. The *M. avium* species includes four closely related subspecies, *M. avium* subsp. *paratuberculosis*, the etiologic agent of Johne's disease or paratuberculosis in ruminants [7]. *M. avium* subsp. *avium* and *M. avium* subsp. *silvaticum*, responsible for avian tuberculosis and infection in wood pigeons, respectively [8] and *M. avium* subsp. *hominissuis*, which is usually isolated from pigs but can also be implicated in human infections [9]. Some recently discovered species are very close to *M. intracellulare* and are termed *M. intracellulare* subsp. *chimaera intracellulare* complex (MCIC) [10][11]. *M. intracellulare* and *M. intracellulare* subsp. *chimaera* are associated with infections in humans. *M. intracellulare* is mainly implicated in pulmonary infections, and *M. intracellulare* subsp. *chimaera* [12], was recently associated with fatal infections after cardiac surgery [13]. MAC can be identified by using DNA probes, luminescent systems, DNA sequencing of *rpoB*, *hsp65* and the 16S–23S Intergenic region, or identification of specific insertion sequences [10]. GenoType NTM-DR, a new commercial diagnostic assay, allows differentiation between three MAC species, *M. avium*, *M. intracellulare*, *M. intracellulare* subsp. *chimaera*, as well as identification of subspecies within the *Mycobacterium abscessus* complex [14]. Mass spectrometry has also been recently proposed as a useful tool to identify these NTM at the species level [15].

Several studies have shown that pathogenic mycobacteria use the protein or proteoglycan component of the extracellular matrix (ECM) for adherence and invasion of the host [16]. One of the best characterized mycobacterial adhesins is the heparin-binding hemagglutinin (HBHA), initially identified in Mycobacterium tuberculosis and Mycobacterium bovis bacillus Calmette-Guérin (BCG) [17][18]. However, HBHA-like molecules are also present in many other mycobacteria, both pathogenic and nonpathogenic [19][20][21][22]. HBHA is located on the surface of the mycobacteria and mediates binding of the bacilli to epithelial cells and fibroblasts [18] by interacting with sulfated glycoconjugates present on the surfaces of host cells [23]. It also plays a role in the dissemination of M. tuberculosis from the lungs to deeper tissues [24] and has shown promise as a diagnostic target for the detection of latent tuberculosis in humans [25][26][27][28].

Laminin and collagen in the lung also promote adherence to ECM-binding mycobacteria, and mycobacterial laminin-binding proteins (LBP) involved in adherence of mycobacteria to host cells have been identified and characterized. LBP was initially described to play a role in the interaction between *Mycobacterium leprae* and Schwann cells [29][30][31]. LBP, also referenced to as Lbp/Hlp [32][33], Mdp1, the Mycobacterial DNA-binding protein 1 [30] and hupB the mycobacterial histone-like protein are conserved in mycobacteria, including MAC [32].

2. Adherence of M. intracellulare to Epithelial Cells is Modulated by Heparin and Laminin

Previous reports have shown that adherence of mycobacteria to epithelial cells can be modulated by the addition of an extracellular matrix component [18][32]. Using the recombinant *M. intracellulare* strain, we tested whether soluble exogenous heparin or laminin can affect the cytoadherence of *M. intracellulare* to A549 epithelial cells. As observed qualitatively by fluorescence microscopy in Figure 1A, the recombinant *M. intracellulare* (green) is able to adhere to the A549 epithelial cells, stained with Blue Evans (red). Adherence is inhibited by the addition of heparin and enhanced in the presence of laminin. Quantification of *M. intracellulare* adherence by luciferase assay indicated that this adhesion to epithelial cells was significantly decreased from 50 to 60% in the presence of exogenous heparin but increased from 35 to 50% in the presence of exogenous laminin (Figure 1B). The diagram in Figure 1C explains how heparin and laminin can modulate the adhesion of bacteria to cells. The addition of heparin decreases the adhesion of bacteria to cells because it represents targets in competition with the heparin present in cell membranes. Conversely, the addition of laminin will indirectly increase the adhesion of bacteria to the cells via the laminin receptor (Figure 1C).

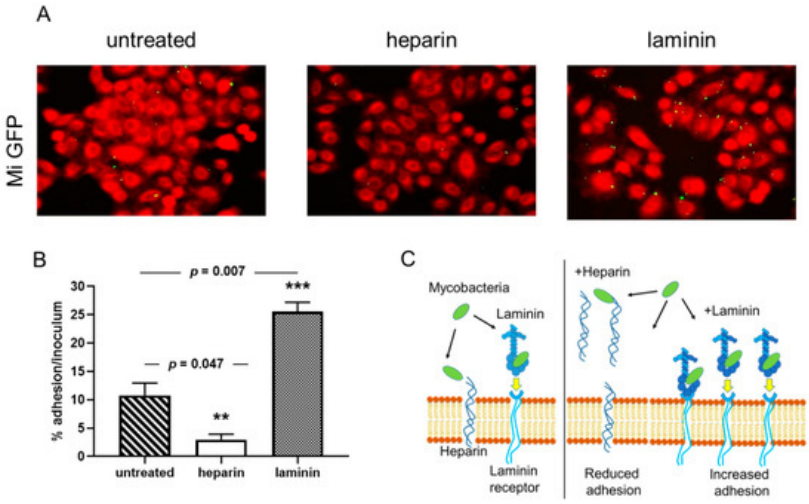

Figure 1. Cytoadherence of *M. intracellulare* ATCC13950 to A549 epithelial cells inhibited heparin and increased by laminin. A549 cells were infected by Green fluorescent protein (GFP)- and luciferase-producing *M. intracellulare* ATCC13950 in the presence or absence of heparin or laminin. (**A**) Fluorescence microscopy analysis of the A549 cells infected by *M. intracellulare* /GFPlux (green). The samples were fixed with PFA and stained with Evans Blue (red). Images taken with 40× objectives represent the overlay of Evans Blue and GFP signals. (**B**) Quantification of *M. intracellulare* /GFPlux adherence by luciferase assays. The percentages of adhesion were calculated by the formula (cell-associated RLU/RLU of the inoculum) × 100. The graph shows the averages of triplicate samples from one representative of three independent experiments. ** $p < 0.05$; *** $p < 0.01$. The error bars represent the standard deviation. (**C**) The diagram gives an illustration of how bacteria bind to cells, either directly on the heparin present on the surface of cells or via laminin which then binds to its cell receptor. In the presence of exogenous heparin or laminin, the adhesion of the bacteria is inhibited or increased.

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