Inhibition of adherence of Mycobacterium avium complex and Mycobacterium tuberculosis to fibronectin on the respiratory mucosa

A.M. Middleton\textsuperscript{a}, M.V. Chadwick\textsuperscript{a}, A.G. Nicholson\textsuperscript{a}, A. Dewar\textsuperscript{a}, R.K. Groger\textsuperscript{b}, E.J. Brown\textsuperscript{c}, T.L. Ratliff\textsuperscript{d}, R. Wilson\textsuperscript{a,*}

\textsuperscript{a}Host Defence Unit, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK
\textsuperscript{b}Washington University, St Louis, USA
\textsuperscript{c}University of California, San Francisco, USA
\textsuperscript{d}University of Iowa, IA, USA

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Summary Mycobacterium species adhere to the respiratory mucosa via mucus and fibronectin of extracellular matrix exposed by damaged epithelium. We have investigated whether inhibiting adherence to fibronectin influences subsequent infection of human respiratory tissue by Mycobacterium avium complex and Mycobacterium tuberculosis. Human respiratory tissue was pretreated with mycobacterial fibronectin attachment proteins prior to infection with M. avium complex and M. tuberculosis and the number of recoverable bacteria over time was compared to untreated controls. Inhibition significantly reduced recovery of M. avium complex at 15 min (P = 0.02), 7 days (P = 0.04), and 14 days (P = 0.03); whereas recovery of M. tuberculosis was only reduced at 15 min (P = 0.01) and not at later timepoints. We conclude that M. avium complex and M. tuberculosis infection of the mucosa proceeds by different mechanisms, since M. tuberculosis infection is independent of fibronectin adherence.

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Introduction

Mycobacterium avium complex (MAC) and Mycobacterium tuberculosis (MTB) both cause pulmonary disease in humans. MAC is less virulent than MTB, and is generally referred to as an opportunistic or facultative pathogen. Although MAC infections do occur in patients without any respiratory history, most patients have prior lung damage. In these cases, the primary site of infection may be the airway mucosa. In contrast, MTB is an obligate human pathogen transmitted from patient-to-patient in aerosols of respiratory secretions and the primary route of infection of MTB is regarded as via interaction with the alveolar macrophages. Little is known about the virulence factors of MAC and MTB and whether they possess any that are specifically relevant to mucosal infections. We have previously investigated the role of adherence to fibronectin in the interaction of mycobacteria with the respiratory mucosa.\textsuperscript{1} In these studies, mycobacteria adhered to fibronectin in extracellular matrix (ECM), as well as...
mucus with a fibrous appearance. They also adhered rarely to healthy unciliated epithelium, degenerated extruded epithelial cells and open tight junctions between epithelial cells.\(^2\)\(^3\)

There have been a number of reported mechanisms of mycobacterial attachment to host cells, including heparin-binding haemagglutinin interaction with epithelial cells and at least five mycobacterial components that bind with HEp-2 cell extracts.\(^4\)\(^5\) Two distinct mycobacterial proteins or protein complexes, fibronectin attachment protein (FAP) and proteins of the antigen 85 complex, are involved in mycobacterial attachment to fibronectin.\(^3\)\(^6\) The ability to bind fibronectin is highly conserved in mycobacteria.\(^3\)\(^7\) We have previously shown that pre-incubation of MAC and MTB with soluble fibronectin and pre-incubation of tissue with FAP and/or antigen 85B protein (a85B) resulted in significant inhibition of the bacterial adherence to fibronectin exposed in areas of damage on the mucosal surface of organ cultures of human respiratory tissue.\(^1\)\(^2\) Antigen 85B protein, was selected for these experiments as it has been shown to be simultaneously associated with the bacterial cell wall as well as being secreted.\(^8\)

We have also previously studied the interaction of MAC and MTB with the respiratory mucosa over 14 days where we showed that MAC increased in numbers extracellularly on the mucosal surface whereas MTB was lost from the surface, but invaded the organ culture and multiplied within the tissue.\(^9\)\(^10\)\(^11\) We have now investigated the effect of inhibiting adherence to fibronectin with FAP and a85B combined on MAC and MTB infection of human respiratory mucosa in an organ culture model with an air interface incubated over 14 days. We hypothesized that the interaction of MAC with fibronectin may be a more important pathogenic mechanism for MAC than MTB, and that by inhibiting adherence we may be able to reduce or prevent infection of the organ culture.

**Materials and methods**

The organ culture using human nasal turbinate and bronchial tissue has been described previously.\(^1\)\(^10\)\(^11\) Each experiment required 12 pieces of dissected tissue from the same donor. Six experiments were performed each with tissue from a different donor. Tissue was either pre-incubated with sterile PBS or with 100\(\mu\)g/ml *M. avium* FAP (amino acids 269–292) and 100\(\mu\)g/ml purified *M. bovis* BCG a85B together by gently pipetting onto the mucosal surface. Tissue was incubated at 37°C for 15 min and then washed (× 3) by gently pipetting 100\(\mu\)l of PBS onto the mucosal surface. A clinical strain of MAC\(^1\) and MTB H37R\(_{\text{V}}\) (ATCC 27294) were used. Washed bacteria resuspended in 100\(\mu\)l of sterile PBS of which 2\(\mu\)l containing approximately 1 \(\times\) 10\(^8\) cfu were inoculated onto each organ culture. Viable counts were performed and there was no significant difference between the inocula of the different experiments. Tissues were incubated at 37°C in 5% CO\(_2\) in air for 15 min, 7 days and 14 days. Organ cultures were then washed by pipetting 100\(\mu\)l (X3) of sterile PBS onto the tissue in order to remove unattached bacteria. Then each of the four edges of the organ culture were touched with a sterile loop and plated onto Columbia agar base (Lab M, Bury, UK) containing 5% defibrinated horse blood and Löwenstein–Jensen (L–J) medium (Royal Brompton Hospital, Microbiology Department, London, UK), in order to assess the sterility of the control organ cultures and the purity of the mycobacterial growth in the infected organ cultures. Tissue was removed from the organ culture taking care to remove all surrounding agar. One hundred microlitre of sterile PBS was placed in a sterile-graduated centrifuge tube and the meniscus was marked on the side of the tube. Tissue was placed in the PBS and the displacement volume was estimated by the amount of PBS required to be removed to return the meniscus to the marked level. The tissue was then placed in 1 ml of sterile PBS in a homogenizer grinder tube and homogenized three times for 10 s, with a 10 s interval between each spin to prevent temperature build-up in the tissue/PBS due to friction. Ten-fold dilutions of the homogenate were performed down to 10\(^{-6}\). Viable counts were performed and the number of bacteria recovered per microlitre of tissue was calculated using the displacement volume of the tissue. The number of MAC and MTB recovered from tissue pre-incubated with PBS was compared to the number recovered from tissue pre-incubated with FAP and a85B at the three incubation times. The number of bacteria recovered at different time intervals were analysed using Wilcoxon’s matched signed rank test, P-values of ≤ 0.05 were judged to be significant. All experiments were coded and randomized prior to analysis making the observer blind to the identity of each individual piece of tissue.

**Results**

The results at 15 min reflect surface adherence, whereas 7 and 14 days reflect a mixture of surface
infection and tissue invasion. The figure shows the mean number of bacteria recovered from macerated organ culture tissue (cfu/µl) incubated for 15 min, 7 and 14 days after pre-incubation of tissue with either PBS or FAP and a85B together. The numbers of MAC recovered from tissue pre-incubated with FAP and a85B were reduced by 89.1% ($P = 0.02$), 88.8% ($P = 0.04$) and 86.7% ($P = 0.03$) after 15 min, 7 and 14 days incubation, respectively, compared to tissue pre-incubated with PBS. Therefore, inhibition of MAC adherence to fibronectin substantially reduced the level of MAC infection of the organ culture. The numbers of MTB recovered from tissue pre-incubated with FAP and a85B were reduced by 92.7% ($P = 0.01$), but at later time points there was no significant difference between organ cultures incubated with either PBS or FAP and a85B together. There was a 35.5% ($P > 0.05$) and 30.7% ($P > 0.05$) reduction in the FAP/a85B incubated tissue at 7 and 14 days incubation, respectively (Fig. 1).

**Discussion**

The significant reduction in MAC and MTB adhering to tissue pre-incubated with FAP and a85B after only 15 min incubation was the same as shown in our previous studies. In these studies, SEM showed significant inhibition of mycobacterial adherence to ECM, the major site of adherence. The results of the extended incubation times, 7 and 14 days, showed that the numbers of MAC recovered were still significantly reduced and the large rise in MAC numbers recovered at 14 days, as seen in the untreated control experiment, did not occur. It may be that a critical number of MAC are required before rapid multiplication can occur, similar to the principle of ‘quorum sensing’ reported for other bacteria. These results show that MAC infection of the organ culture is dependent upon initial adherence to fibronectin. Our previous studies show that bacteria replicate at these sites to form discrete colonies. Therefore, adherence to fibronectin is an important pathogenic mechanism for MAC and could possibly provide an opportunity to interrupt the infectious process of binding could be inhibited in vivo.

However, the number of MTB recovered at 7 and 14 days were no different compared to untreated control tissue. Therefore, in contrast to MAC, inhibition of adherence to fibronectin does not reduce MTB infection of the organ culture. This is likely to be because MTB infection proceeds via invasion of the mucosa, using mechanisms independent of FAP and a85B, such as the heparin-binding haemagglutinin described by Menozzi et al.

This study highlights differences in pathogenic mechanisms of MAC and MTB with the respiratory mucosa, despite similarities in their initial interaction. Inhibition of MAC adherence to fibronectin, for example by immunization with fibronectin attachment proteins, could reduce mucosal infection by MAC.

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References


