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*Letter to the Editor*

# Curli pili affect the intracellular survival of *Mycobacterium tuberculosis*

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Dear Editor,

Tuberculosis (TB) is one of the top ten causes of global mortality, and was responsible for 1.8 million deaths in 2015 [1]. The aetiological agent, *Mycobacterium tuberculosis*, invades, replicates, and survives within host macrophages and epithelial cells [2]. In order to enable the spread of infection, the bacilli must exit the infected host cells, possibly as a result of host cell death mechanisms [3].

*M. tuberculosis* curli pili (MTP), encoded by the *mtp* (*Rv3312A*) gene, mediate adhesion to and invasion of macrophages [4] and epithelial cells [5]. In the present work, the role of MTP in the host-pathogen interaction was further explored, by assessing its contribution to host cell cytotoxicity and bacterial intracellular survival.

Bacterial inocula of the *M. tuberculosis* V9124 wild-type, MTP-deficient Δ*mtp* mutant [6], and MTP-overexpressing *mtp*-complemented [6] strains were prepared as previously described [5]. The THP-1 monocytic and A549 epithelial cell lines were maintained and seeded as described before [4,5], with the exception that $1 \times 10^5$ cells were seeded in 96-well plates. The mammalian cells were infected with the bacterial suspensions for 4 hours, at a multiplicity of infection of 1–5, after which the monolayers were washed and fresh cell culture medium added.

At 5 days post-infection, cytotoxicity was quantified by the measurement of dead-cell protease activity, using the CytoTox-Glo Cytotoxicity Assay (Promega, Leiden, Netherlands), according to the manufacturer's instructions. Colony-forming units of intracellular *M. tuberculosis* were determined, using previously described methods [4], at 4 hours and 5 days post-infection, to compare the intracellular survival of the strains. Data were analyzed by one-way ANOVA, using SPSS version 24 software (IBM SPSS, Chicago, IL, USA). Significance was accepted at $P < 0.05$. GraphPad Prism version 7.03 software (GraphPad Software, La Jolla, CA, USA) was used for the production of the image.

The MTP-deficient and MTP-proficient strains induced similar levels of cytotoxicity in both macrophages (Figure 1A) and epithelial cells (Figure 1B). This finding indicates that unlike pili of some bacteria, e.g., *Pseudomonas aeruginosa* [7], MTP does not impact host cell death pathways.

The intracellular survival rate of the MTP-deficient mutant strain in macrophages was significantly lower than that of the MTP-proficient strains (Figure 1C). This is consistent with studies in other microorganisms, which have suggested that pili promote bacterial resistance to phagocytic killing [8,9].

Conversely, the MTP-deficient mutant strain displayed a significantly higher survival rate within epithelial cells, compared with the MTP-overexpressing complemented strain (Figure 1D). Although not meeting statistical significance, the mutant strain displayed on average a higher survival rate in epithelial cells, compared with the wild-type (Figure 1D). Epithelial cells provide a more favourable intracellular environment for *M. tuberculosis* replication, compared with the bactericidal mechanisms of macrophages [10]. This could be responsible for the differential results obtained between these two host cell types, but further investigations are required to better explain this phenomenon.

In conclusion, whilst MTP are not associated with host cell cytotoxicity, they do affect the intracellular survival of *M. tuberculosis*. Overall, this report provides further evidence on the importance of MTP in *M. tuberculosis* pathogenesis, and its potential as a target for new TB control strategies.

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**Figure 1.** The role of MTP in cytotoxicity (A-B) of and survival (C-D) in macrophages and epithelial cells, respectively. Host cells were infected with the wild-type, Δ*mtp* mutant, and *mtp*-complemented strains for 4 hours. At 5 days post-infection, dead and live host cells were quantified, and the percentage cytotoxicity was calculated. Intracellular survival is depicted as a ratio of the colony-forming units at 5 days post-infection, relative to that at 4 hours post-infection. Error bars represent standard deviation.