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Tyrosine phosphatase MptpA of *Mycobacterium tuberculosis* inhibits phagocytosis and increases actin polymerization in macrophages

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Abstract

Protein tyrosine phosphatases from several microorganisms have been shown to play a role as virulence factors by modifying the phosphorylation/dephosphorylation equilibrium in cells of their host. Two tyrosine phosphatases, MptpA and MptpB, secreted by *Mycobacterium tuberculosis*, have been identified. Expression of MptpA is upregulated upon infection of monocytes, but its role in host cells has not been elucidated. A eukaryotic expression vector containing the *mptpA* cDNA has been transfected into macrophages. We report that MptpA reduced phagocytosis of mycobacteria, opsonized zymosan or zymosan, but had no effect on phagocytosis of IgG-coated particles. We also noted that the presence of F-actin at the surface of phagosomes containing opsonized zymosan was significantly increased in cells expressing MptpA. In the presence of recombinant MptpA, the process of actin polymerization at the surface of isolated phagosomes was increased; this was not the case in the presence of the phosphatase-dead mutant $MptpA_{C11S}$. MptpA had no effect when IgG-coated particles were present inside isolated phagosomes. These results indicate that, like other tyrosine phosphatases of pathogens, MptpA plays a role in phagocytosis and actin polymerization. However, MptpA had no effect on IgG particles, suggesting that its putative substrate(s) is not linked to the signaling pathways of Fcγ receptors.

Keywords: Tyrosine phosphatase; *Mycobacterium tuberculosis*; Macrophage; Phagocytosis; Actin

1. Introduction

Tuberculosis is the most important cause of death from infectious diseases. Our knowledge of how *Mycobacterium tuberculosis* enters the host cell, or macrophage, and circumvents bactericidal responses remains elusive. It is clear that *M. tuberculosis* and other pathogenic species such as *M. avium* and *M. kansasii*, which often cause severe infections in immunodepressed and AIDS patients, are internalized into phagosomes which remain refractory to fusion with lysosomes [2,29]. This is probably part of the survival strategy of these bacteria so as to avoid acidic pH, proteases and bactericidal proteins found in lysosomes. It is interesting to note that both pathogenic and nonpathogenic mycobacteria remain inside phagosomes which do not fuse with lysosomes at early time points post-infection (<1 h) [4]. To do so, they are internalized through special receptors [4,38,41]. At longer time points post-infection, phagosomes containing nonpathogenic mycobacteria fuse with lysosomes [29]. In contrast, phagosomes indefinitely resist fusion when they contain pathogenic strains, indicating that these mycobacteria have the capacity to control some host cell defenses. This activity requires live bacteria and putative virulence factors.

Among strategies developed by bacteria to circumvent host cell defenses, it has been shown that Gram-negative bacteria such as *Yersinia* sp. [20,23,42,48], *Salmonella ty-*

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phimurium [22,28] and *Helicobacter pylori* [44] are equipped with tyrosine phosphatases dedicated to host proteins [19,27]. For example, the virulence factor YopH of *Yersinia* is a tyrosine phosphatase which disturbs cytoskeleton dynamics and inhibits the phagocytic process disarming the immune cell system [7].

Sequence analysis of the *M. tuberculosis* genome revealed the presence of two genes *mptpA* and *mptpB* encoding low molecular weight phosphotyrosine phosphatases MptpA and MptpB (http://www.genolist.pasteur.fr/TubercuList) [11]. These two enzymes have already been expressed as recombinant proteins, and shown to possess phosphotyrosine activity and to lack activity toward serine and threonine-phosphorylated proteins [31]. MptpA is released by *M. tuberculosis* [15,31] and, upon infection of human monocytes, the expression of MptpA is triggered in *M. bovis* BCG but not in *M. smegmatis* [15]. Moreover, a mutant strain lacking *mptpB* presents impaired ability to survive in activated macrophages or guinea pigs [45]. Since there is no counterpart protein tyrosine kinase in the *M. tuberculosis* genome, we expect that the role of tyrosine phosphatases is not dedicated to regulating the phosphorylation state of mycobacterial proteins, but rather to acting on host cells. The membrane of phagosomes containing mycobacteria has been shown to become permeable to high molecular weight molecules [47] and recently, mycobacteria free in the cytoplasm of macrophages have been described [46] suggesting that proteins secreted by mycobacteria could easily be released in the host cell cytosol. In fact, a serine/threonine kinase secreted by *M. bovis* BCG and present in the cytoplasm of infected cells has recently been shown to affect the fusion of lysosomes with phagosomes [49]. We thus decided to examine the role of the *M. tuberculosis* tyrosine phosphatase MptpA in macrophages. We report that MptpA inhibits the phagocytic process of mycobacteria or zymosan without affecting ingestion of immunoglobulin G-coated particles. In addition, it enhances the presence and the polymerization of actin at phagosomes.

2. Materials and methods

2.1. Bacteria, plasmid construction and mutagenesis

The primers used in this study and listed in Table 1 were provided by Sigma–Genosys Ltb. (Haverhill, UK). The *E. coli* strain was grown and maintained in LB medium at 37 °C. Restriction enzymes and T4 DNA ligase were used as recommended by the manufacturer (Promega, Charbonnières, France).

The 492-bp *mptpA* gene fragment, with appropriate sites at both ends, was synthesized by PCR amplification using *M. tuberculosis* H37Rv genomic DNA as a template, primer pair 1/2 (Table 1) and Pfu polymerase purchased from Promega. This DNA fragment was restricted by *Kpn*I and *Pst*I and ligated into the *E. coli* expression vector pQE-30 generating His_6 fusion proteins, thus yielding pQE-30-*ptpA* (Table 2). A plasmid designed to express MptpA in mammalian cells was constructed by PCR amplification using *M. tuberculosis* H37Rv genomic DNA as a template and primer pair 7/8 (Table 1). The resulting DNA fragment was restricted by *Kpn*I and *Xba*I and ligated into vector pcDNA3 digested by the same enzymes, thus yielding pcDNA3-*ptpA* (Table 2). PCR products and plasmid DNA fragments were purified using the QiaexII kit (Qiagen, Courtaboeuf, France).

According to the conserved catalytic site of low molecular weight phosphatases [50], site-directed mutagenesis of MptpA was performed [35] on Cys_{11} to Ser by PCR amplification using pQE-30-*ptpA* as template and oligonucleotide

Table 1
Primers used in this study

Primer [a]	5′ to 3′ sequence [b,c]
1 (+)	TAT*GGTACC*TCTGATCCGCTGCACGTCACATTC
2 (–)	TAT*CTGCAG*AACTCGGTCCGTTCCGCGCGAGACG
7 (+)	TAT*GGTACC*ATGTCTGATCCGCTGCACGTCACAT
8 (–)	TAT*TCTAGA*TCAACTCGGTCCGTTCCGCGCG
3 (+)	TAT*GGTACC*TCTGATCCGCTGCACGTCACATTCGTTAGTACGGGCAACATCGCAAC

[a] Forward and reverse primers are represented by plus (+) or minus (–), respectively.
[b] Restriction sites are *italicized*.
[c] The bases mutated from those present in the wild type are underlined.

Table 2
Plasmids used in this study

Plasmids	Description	Reference or source
pQE-30	*E. coli* expression vector generating His_6 fusion proteins for overexpression	Qiagen
pQE-30-*ptpA*	pQE-30 with an 492-bp *Kpn*I/*Pst*I fragment encoding entire MptpA protein	This study
pQE-30-$ptpA_{C11S}$	pQE-30 with an 492-bp *Kpn*I/*Pst*I fragment encoding entire $MptpA_{C11S}$ mutant	This study
pcDNA3	Mammalian cell expression vector	Invitrogen
pcDNA3-*ptpA*	pcDNA3 with an 492-bp *Kpn*I/*Xba*I fragment encoding entire MptpA protein	This study