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Interactions between an *M. tuberculosis* strain overexpressing *mtrA* and mononuclear phagocytes

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ABSTRACT

Purpose: It was previously shown that the bacterial two-component regulatory signal transduction (2CR) system MtrAB may be associated with the ability of *M. tuberculosis (Mtb)* to survive in macrophages. In the present work *Mtb* mutants: Rv-78 with overexpression of *mtrA* and Rv-129 with elevated level of phosphorylation-defective MtrA were used for further investigation of the potential influence of the MtrAB system on *Mtb* interaction with human monocytes.

Material/Methods: Flow cytometry was used to determine the expression of MHC class II molecules. The expression of genes for inducible nitric oxide synthase (iNOS) and cathepsin G was quantified by RT-PCR. The association of *Mtb* strains with Rab5 and Rab7 positive vacuoles was investigated applying confocal microscopy. IL-10 and IL-12 secretion by monocytes as well as the *Mtb* susceptibility to cathepsin G were investigated.

Results: Mutation-carried and wild type *Mtb* strains inhibited MHC class II expression on monocytes to a similar extent. Monocyte stimulation with mycobacteria led to the increased production of IL-10 but no detectable amounts of IL-12 or NO were observed. Expression of the gene for iNOS was not detected while that for cathepsin G was shown, however its intensity was not associated with MtrA mutation. *Mtb* mutant strains were more effectively enclosed in phagosomes containing the late endosome marker Rab7 as compared to the control.

Conclusions: The results may confirm the importance of the MtrAB system in mycobacterial capacity for successful survival in phagocytes, especially in the context of high degree of colocalization of *Mtb* Rv-78 to mature phagosomes.

Key words: *M. tuberculosis*, MtrA, mononuclear phagocytes, MHC class II, phagosome

INTRODUCTION

Mycobacterium tuberculosis (*Mtb*) is a facultative intracellular bacterium which has evolved sophisticated mechanisms to evade antimicrobial processes of macrophages. The balance between the bactericidal potency of macrophages and the evasion strategies of *M. tuberculosis* regulates the course of mycobacterial infection. However, there is an urgent need for a better understanding of the mechanisms that allow mycobacteria to effectively avoid host immune mechanisms and for the identification of *M. tuberculosis* proteins which may serve as effective drug targets. The bacterial two-component regulatory signal transduction systems (2CRs) play an important role in the intracellular survival of *M. tuberculosis*. The systems consist of pairs of sensor and regulatory proteins [1-3] which detect signals from the external environment, transfer them to the bacterial cells resulting in transcriptional responses [4]. The *Mtb* genome

possesses 11 pairs of genes identified as those encoding 2CR systems [5], among them only the MtrAB system is absolutely essential for mycobacterial growth [6-8]. A previous study on *M. tuberculosis* mutant Rv-78, characterized by an elevated intracellular level of the MtrA regulator protein of the MtrAB system, showed that overexpression of the *mtrA* gene results in reduced *Mtb* survival in the monocytic THP-1 cell line and blood monocyte-derived macrophages. This phenomenon was not observed for the *Mtb* strains with overexpression of individual components of other 2CR systems. This allows suggesting that overexpression of *mtrA* gene is, at least partially, responsible for the attenuation of the mutant growth not only *in vitro* but also in the lungs and spleen of infected mice [9]. Moreover, the fact that MtrA protein targets the *dnaA* promoter (an essential replication initiator gene) and that *dnaA* transcription *in vivo* is promoted in the MtrA-phosphorylation dependent manner [9] confirms the importance of the MtrA overproduction in mycobacterial virulence. An *M. tuberculosis* strain showing an elevated level of phosphorylation-defective MtrA (Rv-129) did not multiply in macrophages and murine lungs as effectively as parental wild-type H37Rv (Rv-wt), however, the expression level of *dnaA* was similar in both strains [9]. It is interesting that *M. bovis* BCG also exhibits prominent upregulation of the MtrA protein during growth within macrophages [10-12].

In the present work we further explored a possible influence of the MtrAB system on the fate of ingested mycobacteria within macrophages. It has been reported that the survival of tuberculosis bacilli in macrophages is associated with: a) disturbances in the early/late endosome transfer or maturation [13-17], b) reduced acidification of mycobacterial phagosomes [18, 19], c) downregulation of MHC class II molecule expression critical for antigen presentation [14, 20, 21], and d) inefficient displacement of iNOS (inducible nitric oxide synthase) in the immediate vicinity of mycobacterial phagosomes [22, 23]. The results of this study show that the MtrAB system may influence the intracellular survival of *Mtb.* This may be accomplished by affecting trafficking of the bacteria to mature phagosomes, as mycobacterial phagosomes containing the Rv-78 mutant more effectively recruited late endosome marker Rab7 than did phagosomes containing the Rv-wt strain. No significant influence of *mtrA* expression disturbance on the susceptibility of mycobacteria to cathepsin G or on the level of MHC class II restriction was observed.

MATERIAL AND METHODS

Mycobacterial strains and culture conditions

The laboratory, virulent wild-type strain of *M. tuberculosis* H37Rv (Rv-wt), *M. tuberculosis* Rv-wt strain expressing green fluorescent protein (Rv-wt GFP), *M. tuberculosis* Rv-78 – mutant overexpressing regulatory protein MtrA (Rv-78), *M. tuberculosis* Rv-78 expressing GFP (Rv-78 GFP), *M. tuberculosis* Rv-129 mutant producing an elevated level of phosphorylation-defective MtrA (Rv-129), *M. tuberculosis* Rv-129 expressing GFP (Rv-129 GFP), attenuated strain *M. bovis* BCG and *M. bovis* BCG expressing GFP (BCG-GFP) were grown in Middlebrook 7H9 medium (Difco, Becton Dickinson) enriched with 10% oleic acid-albumin-dextrose-catalase (OADC, Becton Dickinson), 0.05% Tween 80. Antibiotics: hygromycin (Sigma) at 50μg/ml for Rv-78 and Rv-129, kanamycin (Sigma) at 25μg/ml for *M. bovis* BCG GFP, and both of them for Rv-78 GFP and Rv-129 GFP were used. *M. tuberculosis* H37Rv and *M. bovis* BCG were a gift kindly provided by the Institute of Tuberculosis and Lung Diseases, Warsaw, Poland, whereas all *M. tuberculosis* mutants and *M. bovis* BCG-GFP were generous gifts from the University of Texas Health Science Center at Tyler, USA. Bacterial growth was estimated by reading the optical density at 600nm.

THP-1 cells culture

The monocyte-like cell line THP-1 (DSMZ, #ACC16, Germany) was grown as previously described [24] with minor modifications. Cells were maintained in RPMI-1640 medium (Sigma) supplemented with 10% fetal bovine serum, FBS (PAA, Austria), 2mM L-glutamine and 1mM sodium pyruvate and cultured at 37°C, 5% CO_2. THP-1 cells were differentiated into adherent, well-spread macrophages by the addition of 50 nM phorbol myristate acetate, PMA (Sigma) and incubated for 48h before the experiment. Adhered macrophages (5×10^5/well) were then washed three times with plain RPMI medium and next the cells (in culture medium) were exposed to bacteria for 3h at MOI (multiplicities of infection) of 5:1 for RT-PCR experiments or 20:1 for confocal microscopy. After that, noningested bacteria were removed by washing the cell monolayer (three times) with plain RPMI 1640 medium. Cells were resuspended in RPMI 1640 medium supplemented with 2% FBS and cultured 24h or 72h, respectively.

Monocyte isolation and culturing

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood of healthy adult volunteers by density sedimentation over LSM 1077 separation medium (PAA, Austria). Briefly, blood drawn into heparinized vacutainer tubes (Kima, Italy) was diluted 1:1 with RPMI 1640 medium (Sigma), next laid over the LSM 1077 medium (4:3) and centrifuged (1200×*g* for 20 min at room temp.). The obtained interphase containing PBMC was collected, washed twice with RPMI 1640, and then monocytes were isolated using the negative immunomagnetic separation MACS system (Miltenyi Biotech, Germany) according to the manufacturer's instructions. 5×10^5 monocytes in RPMI medium (supplemented with 10% FBS and L-Glu) were seeded onto a 12-well tissue culture plate and infected with different strains of *Mycobacterium* at MOI of 5:1 for 3h at