Ex Vivo Fitness of MTB Correlates With Lysozyme Sensitivity in the East-African Indian Lineage

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Background

Virulence factors (VFs) facilitate persistence of *Mycobacterium tuberculosis* (MTB) and are implicated in the recent emergence of new human isolates. Some VFs are lineage specific and thus difficult to identify or study; however, understanding of them is essential to the development of new drugs and vaccines against MTB. We sought to identify new VFs/markers of virulence in Central Asian (CAS) clinical isolates.

Methodology

Three large-cluster CAS isolates, designated C6, C14 and C72, from the East-African Indian (EAI) lineage were studied in guinea pigs and human monocyte-derived macrophages (MDMs). References were outbreak isolates E and L1, from East-Asian and Euro-American lineages respectively and in MDMs, unique CAS isolate C40, H37Rv/Ra, and BCG. Pathogenicity scores (PS) were used to assess the severity of lesions in the lungs of infected animals. PS are derived from blind, yet subjective, scoring of consolidation, necrosis and calcification. Colony forming units (CFU) recovered from spleen samples of infected animals were used to measure isolate dissemination within host. MTB intracellular replication ex vivo was measured by qPCR for bacterial gene SigA relative to human gene TERT. Whole-genome sequencing was performed on all CAS, Illumina reads mapped to a corrected version of the H37Rv reference genome using SMALT and single nucleotide polymorphisms (SNPs) identified with SAMtools. For lysozyme (LYZ) sensitivity testing, CAS isolates and reference strains were plated on 7H10 agar plates with increasing concentrations of LYZ and CFUs counted at three weeks.

Results

- Differences in PS and CFU were observed in vivo but only differences in fitness ex vivo were statistically significant; C72 was least fit (**Figure 1**).

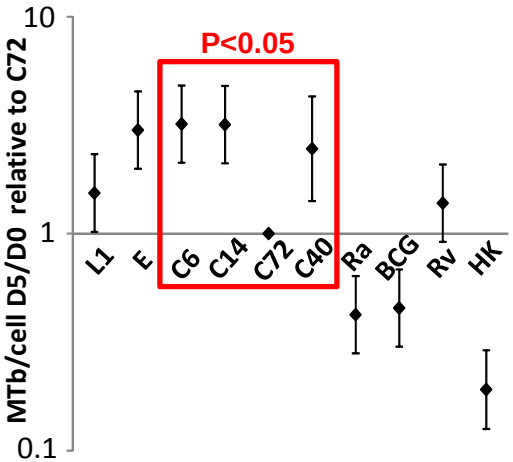


Figure 1. Ex vivo phenotypes of EAI clinical MTB isolates and reference strains in MDMs. MDMs from six donors were infected in duplicate (MOI=10) and lysed at four hours (D0) or five days (D5) post-infection. Bacilli/cell numbers were determined at D0 and D5 on lysates using duplex qPCR for *sigA* and *hTERT* and calculating ratios of *sigA* to *hTERT* copies. Heat-killed (HK) MTB was used as control. The log-transformed ratios of bacilli/MDM estimated at D5 to D0 (D5/D0) was analysed relative to the same ratio for C72 using an analysis of variance; 95% confidence intervals are shown.

- Comparative genomic analysis revealed 123 unique non-synonymous SNPs as candidates for the observed differential phenotype of C72. Of these, nine locate in genes previously associated with fitness ex vivo or dissemination in vivo; three were additionally linked to both acid and LYZ resistance in vitro.
- Accordingly, LYZ sensitivity testing in vitro showed that all isolates were inhibited by increasing concentration of LYZ ($p<0.01$) and C72, the most.

Conclusions

Earlier work linked LYZ sensitivity to MTB fitness and dissemination in vivo, but for Euro-American reference strain H37Rv only. We now describe a similar correlation for EAI clinical isolates. Further work will determine whether genes conferring LYZ resistance are VFs per se.



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