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Disruption of response regulator gene, *devR*, leads to attenuation in virulence of *Mycobacterium tuberculosis*

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Abstract

The *devR-devS* two-component system of *Mycobacterium tuberculosis* was identified earlier and partially characterized in our laboratory. A *devR::kan* mutant of *M. tuberculosis* was constructed by allelic exchange. The *devR* mutant strain showed reduced cell-to-cell adherence in comparison to the parental strain in laboratory culture media. This phenotype was reversed on complementation with a wild-type copy of *devR*. The *devR* mutant and parental strains grew at equivalent rates within human monocytes either in the absence or in the presence of lymphocytic cells. The expression of DevR was not modulated upon entry of *M. tuberculosis* into human monocytes. However, guinea pigs infected with the mutant strain showed a significant decrease in gross lesions in lung, liver and spleen; only mild pathological changes in liver and lung; and a nearly 3 log lower bacterial burden in spleen compared to guinea pigs infected with the parental strain. Our results suggest that DevR is required for virulence in guinea pigs but is not essential for entry, survival and multiplication of *M. tuberculosis* within human monocytes in vitro. The attenuation in virulence of the *devR* mutant in guinea pigs together with DevR-DevS being a bona fide signal transduction system indicates that DevR plays a critical and regulatory role in the adaptation and survival of *M. tuberculosis* within tissues.

Keywords: DevR-DevS two-component system; *Mycobacterium tuberculosis devR* mutant; Attenuation of virulence

1. Introduction

The *Mycobacterium tuberculosis* genome encodes 11 complete two-component systems and seven orphan signal transduction proteins [1]. While four of them, MtrA [2], SenX3-RegX3 [3], TrcS-TrcR [4] and DevR-DevS [5], have been established as authentic two-component systems based on their biochemical properties, their role in regulating various cellular functions is just beginning to be elucidated. MtrA is essential [6] and other response regulators, PhoP, PrrA and MprA, have been implicated in virulence [7], adaptation of tubercle bacilli in an early phase of intracellular growth [8], and the establishment and maintenance of persistent infection [9], respectively. Likewise, our understanding of the environmental cues sensed by these two-component systems is quite preliminary. Bacterial entry into macrophages was reported to lead to alterations in the activity of several response regulator genes [9]. Hypoxia was recognized as a key signal that led to extensive, rapid and dramatic changes in gene expression including the induction of the *devR-devS* two-component system [10].

The DevR-DevS (Rv3133c-Rv3132c) system was first identified in our laboratory [11] by a subtractive hybridization strategy employed with the specific intention of identifying virulence genes of *M. tuberculosis* [12]. The *devR-devS* genes encode a response regulator, DevR, and

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