

***Mycobacterium tuberculosis* specific CD8<sup>+</sup> T cells require perforin to kill target cells and provide protection in vivo**

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Optimal immunity to *Mycobacterium tuberculosis* (Mtb) infection requires CD8<sup>+</sup> T cells, and several current Mtb vaccine candidates are being engineered to elicit enhanced CD8<sup>+</sup> T cell responses. However, the function of these T cells and the mechanism by which they provide protection is still unknown. We have previously shown that CD8<sup>+</sup> T cells specific for the mycobacterial antigens CFP10 and TB10.4 accumulate in the lungs of mice following Mtb infection and have cytolytic activity *in vivo*. Here we determine which cytolytic pathways are used by these CD8<sup>+</sup> T cells during Mtb infection. We find that Mtb-specific CD8<sup>+</sup> T cells lacking perforin have reduced cytolytic capacity *in vivo*. In the absence of perforin, residual cytolytic activity is CD95- and TNFR-dependent, particularly in Mtb infected lung where disruption of both perforin and CD95 eliminates target cell lysis. Importantly, adoptive transfer of immune CD8<sup>+</sup> T cells isolated from WT, but not perforin deficient mice, protects recipients from *M. tuberculosis* infection. We conclude that CD8<sup>+</sup> T cells elicited following Mtb infection can use several cytolytic pathways in a hierarchical manner dominated by perforin-mediated cytotoxicity, but adoptively transferred Mtb-specific CD8<sup>+</sup> T cells specifically require perforin-mediated cytotoxicity to protect animals *in vivo*. In addition, *in vitro* studies suggest that both IFN $\gamma$  and perforin are required for CD8<sup>+</sup> T cell control of bacteria in macrophages. These data show that CD8<sup>+</sup> T cell-mediated protection during Mtb infection requires more than just the secretion of IFN $\gamma$ , and suggest an integrated IFN $\gamma$ /perforin requirement for CD8<sup>+</sup> T cell-mediated protection in Mtb infection.