CD40 SIGNALING IS REQUIRED FOR THE PRIMING OF THE TYPE I RESPONSE IN ACUTE *M. TUBERCULOSIS* AEROSOL INFECTION

Vanja Lazarevic, Amy J Myers, and JoAnne L Flynn
Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh PA 15261

ABSTRACT

To identify the mechanism by which CD4 T cells contribute to protective immunity against *M. tuberculosis*, we examined the requirement for CD40/CD40L interaction in the course of acute infection. The results of this study show that CD40-/- mice succumb rapidly to low doses of *M. tuberculosis* after aerosol infection. While macrophages from CD40-/- and wild-type mice expressed NOS2, and were equally capable of reducing intracellular mycobacteria when incubated with CD4 or CD8 T cells from immune mice *in vitro*, the primary response of CD40-/- mice toward *M. tuberculosis* was significantly impaired. Few CD4 and CD8 T cells in the lungs acquired an activated phenotype, and minimal IFN-γ production was induced in the lymph nodes and lungs of CD40-/- mice until 4 weeks post-infection. Failure to prime a vigorous Th1 type response is the main cause of enhanced susceptibility of CD40-/- mice to *M. tuberculosis*. The impaired development of cell-mediated immunity appears to be related to the inability of CD40-/- dendritic cells to produce sufficient levels of IL-12 in response to *M. tuberculosis* infection. We show that the dependence on CD40 in the generation of protective T cell responses is a function of antigen dose. Systemic infection of mice with a higher dose of *M. tuberculosis* overcame the need for co-stimulation in the induction of protective T cell mediated immunity. In contrast to the CD40-/- mice, CD40Ligand deficient mice were not susceptible to *M. tuberculosis* aerosol infection. This suggests an alternative ligand for CD40 stimulation; our *in vitro* and *in vivo* data support that this ligand is mycobacteria-derived. Both *in vitro M. tuberculosis* infection, as well as recombinant *M. tuberculosis* HSP70, elicited IL-12 production from WT dendritic cells. This response was absent in both CD40-/- dendritic cells and CD40-/- mice, suggesting that *M. tuberculosis* HSP70 serves as an alternative ligand for CD40 *in vivo*. Collectively, our data suggest that stimulation of CD40 on APCs contributes to the generation of effective cell-mediated immunity against *M. tuberculosis*.