***In vivo* administration of soluble gp33-D^b-tetramer induces apoptosis in naïve P14 CD8$^+$ T cells**

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We have previously shown that intraperitoneal injection of soluble tetrameric MHC class I molecules plus cognate peptide (tetramer) results in activation and division of naïve splenic CD8$^+$ T cells. In the present study, we investigated responses in the draining popliteal lymph node (LN) following administration of tetramer into the footpad, and have observed a profound decline in specific T cells that peaks 24 hours post-injection (PI), and is subsequently replaced by the proliferative phase. We hypothesized that this depletion of naïve T cells is due to tetramer-induced apoptosis that occurs shortly after binding of tetramer to the TCR. **METHODS**: Negatively-selected, naïve splenic CFSE-labeled or unlabeled P14, or P14.GFP, CD8$^+$ T cells (TCR transgenic for LCMV gp$_{33-41}$ / D^b) were incubated *in vitro* with tetramer, or adoptively transferred (AT) into C57BL/6 hosts. At various time points after culture or footpad injection, splenocytes or lymphocytes were harvested, stained with Annexin V-PE, propidium iodide (pi), and anti-CD8α mAb, and analyzed by flow cytometry. As a positive control, T cells were treated with 30 Gray of γ-irradiation *in vitro* to induce apoptosis. **RESULTS**: Incubation of P14 splenocytes for four hours with gp33-D^b-tetramer, but not control tetramer, induced apoptosis (Annexin V-PE$^+$ pi$^-$) in a dose-dependent manner. Similarly, *in vivo* administration of 10 μg of gp33-D^b-tetramer induced apoptosis in P14.GFP and CFSE-labeled P14 T cells, but not in host CD8$^+$ T cells, in the draining LN and spleen at 1 hour PI. At the dose and time points studied, injection of the higher avidity C9M-D^b-tetramer induced an equivalent % of cells to undergo apoptosis. **CONCLUSIONS**: Injection of tetramer induces apoptosis in a fraction of naïve CD8$^+$ T cells in an antigen-specific manner shortly after TCR-tetramer binding, which may account for the depletion of AT P14 T cells in the draining lymph node 24 hours PI. Tetramer-induced apoptosis could potentially limit T cell responses to this form of antigen *in vivo.*