

## Surface phenotype of CD8<sup>+</sup> T cells activated by signal one alone *in vivo* depends on the avidity of TCR – peptide/MHC interactions

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Our laboratory has previously demonstrated that stimulation of naïve CD8<sup>+</sup> T cells with soluble tetrameric MHC class I molecules plus cognate peptide (tetramer) is sufficient for priming *in vitro* and *in vivo*. In this study, we characterized the early response ( $\leq 2$  days) of T cells to signal one alone *in vivo*, and investigated the hypothesis that high avidity TCR-peptide/MHC interactions would potentiate effector differentiation. **METHODS:** Splenic P14 CD8<sup>+</sup> T cells (TCR transgenic for LCMV gp33-41 / D<sup>b</sup>) were purified by negative selection, labeled with CFSE, and adoptively transferred (AT) into C57BL/6 hosts. At various time points post-injection (PI) of tetramer into the footpad, spleens and popliteal lymph nodes (LN) were harvested, stained with mAbs for surface markers or intracellular IFN- $\gamma$ , and analyzed by flow cytometry. **RESULTS:** Injection of a saturating dose of gp33-D<sup>b</sup>-tetramer induced a rapid change to an activated surface phenotype (CD25<sup>+</sup> CD44<sup>hi</sup> CD62L<sup>lo</sup> CD69<sup>+</sup>) in all AT P14 T cells in the draining LN within 6 hours. By 24 hours PI, however, <50% of these cells retained a fully-activated surface phenotype. P14 cells in the LN proliferated vigorously, with 1 division at 24 hours, 2 divisions at 36 hours, and 5 divisions at 48 hours. In the spleen and contralateral LN, a smaller % of P14 cells were activated, and the peak magnitude of surface changes and number of cell divisions was less. At 48 hrs PI, tetramer-activated P14 cells from the spleen and draining LN produced IFN- $\gamma$  when re-stimulated with gp33 peptide *in vitro*. Most maximally-divided P14 cells were IFN- $\gamma$ <sup>+</sup>, independent of surface phenotype (CD25<sup>-</sup> or CD25<sup>+</sup>), while cells undergoing no or few divisions responded suboptimally to peptide. When compared to gp33-D<sup>b</sup>-tetramer, injection of the higher avidity C9M-D<sup>b</sup>-tetramer resulted in similar initial binding to P14 cells and activation kinetics. At 48 hours, however, a significantly higher percentage of C9M-D<sup>b</sup>-tetramer-activated T cells retained an activated surface phenotype, although the number of cell divisions and % IFN- $\gamma$ <sup>+</sup> cells was not different between groups. Interestingly, despite robust production of IFN- $\gamma$ , cytotoxic lymphocyte responses could not be detected *in vivo* in P14 T cell-transferred mice injected with either the wild-type or higher avidity peptide-MHC tetramers. **CONCLUSIONS:** High avidity interaction of TCR with signal one *in vivo* results in enhanced production of activated T cells with effector surface phenotype. Further studies are ongoing to determine whether such high avidity interactions improve memory cell output or protective immunity.