

# *In vivo* visualization of CTL responses in lymph nodes

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## Abstract

Cell migration and coordinated cell-cell interactions are hallmark features of the immune system. Recent advances in real-time *in vivo* imaging technology have added a new dimension to our efforts to understand the dynamics and complex interplay of the key cellular players in the steady state and during ongoing immune responses. In particular, multiphoton intravital microscopy (MP-IVM) allows prolonged three-dimensional observations of highly dynamic events that occur hundreds of micrometers below the surface of solid tissues in living animals. Using a newly developed MP-IVM model in mouse popliteal lymph nodes, we have recently dissected the kinetics of activation of naive CD8<sup>+</sup> T cells by antigen-presenting dendritic cells. This priming event occurs as a three-phase process involving distinct stages of cellular interaction. Once the T cells have become activated, they proliferate and differentiate into cytotoxic effector cells (CTLs). Using a modified MP-IVM protocol we have analyzed how CTLs interact with tumor antigen-presenting target cells in the presence and absence of activated CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (Tregs). We observed that CTLs without Tregs killed their targets at a 6.6-fold faster rate than regulated CTLs. Despite this compromised effector activity, regulated CTLs had no defect in proliferation, induction of cytotoxic effector molecules or secretory granules, *in situ* motility, or ability to form antigen-dependent conjugates with target cells. Only granule exocytosis by CTLs was markedly impaired in the presence of Tregs. This selective regulation occurred in the absence of prolonged Treg-CTL conjugation and required CTL responsiveness to TGF- $\beta$ . Moreover, CTLs quickly regained full effector capacity in lymph nodes upon selective removal of Tregs, indicating that Tregs suppress CTL-mediated adaptive immunity *in vivo* by creating a local milieu that permits the acquisition of effector potential, but withholds the license to kill.