Kishimoto *et al.* reply:
We are surprised that Villunger *et al*. did not find a defect in negative selection in the NOD thymus. Our own results are clear-cut and involved more than 30 experiments both *in vitro* and *in vivo*. Moreover, the negative selection deficit we describe for the NOD thymus is unique to this strain (relative to several other autoimmune disease–prone strains) and applies only to semimature medullary T cells and not to immature cortical $CD4^+CD8^+$ cells. Although we cannot explain the disparate results of Villunger *et al.*, four points can be made. First, for the experiments shown, the semimature $HSA^+CD4^+CD8^-$ thymocytes used by Villunger *et al.* to test for negative selection were presumably coated with CD4 and HSA mAbs, thus possibly altering the response of the cells to CD3-CD28 ligation. We avoid this problem by using a panning procedure for cell preparation. Second, to induce negative selection, we used TCRβ mAb and soluble enterotoxin B as TCR ligands. In contrast, Villunger *et al.* relied on the use of CD3ε mAb, which bypasses TCR ligation. The authors state that they used a panning procedure and TCRβ mAb in some experiments, but these data are not presented. Third, the disparity in the results may reflect subtle but distinct differences in the sublines of NOD mice used in the two studies. Fourth, our data are in close agreement with the recent report of Lesage *et al.*[6]; those authors demonstrated a conspicuous deficit in negative selection in the NOD thymus using a TCR transgenic model. Clearly, resolving the controversy will require experimental input from other investigators.

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