Efficient T cell receptor–mediated apoptosis in nonobese diabetic mouse thymocytes

To the editor:

Impaired deletion of autoreactive thymocytes may contribute to the pathogenesis of autoimmune diseases, and it has been reported that diabetes-prone nonobese diabetic (NOD) mice have a defect in thymocyte apoptosis induced by T cell receptor (TCR)-CD3 ligation[1]. Because the pro-apoptotic BH3-only B cell lymphoma/leukemia 2 (Bcl-2) family member Bim is required for thymocyte negative selection[2,3], we wanted to compare TCR-mediated apoptosis between NOD mice with wild-type C57BL/6 and Bim-deficient mice.

We purified immature, semimature and mature thymocytes from NOD and C57BL/6 mice by cell sorting and investigated their sensitivity to spontaneous apoptosis and apoptosis induced by TCR-CD3 ligation in culture. The spontaneous death of immature $CD4^+CD8^+$ thymocytes from NOD mice was consistently higher than that of the corresponding population from C57BL/6 mice (**Supplementary Fig. 1**). TCR-CD3 stimulation by CD3ε monoclonal antibody (mAb; 145-2C11) or TCRβ mAb alone induced apoptosis of immature $CD4^+8^+$ and semimature $CD4^+CD8^-$ heat-stable antigen–positive (HSA^+) NOD and C57BL/6 thymocytes in culture with similarly low efficiency (**Fig. 1** and **Supplementary Fig. 1**). In both strains, TCR-mediated cell killing was enhanced by the addition of CD28 mAb. 'Specific killing' induced by treatment with CD3 mAb (10 g/ml) plus CD28 mAb (37N51; 20 g/ml) was 45 ± 13% for NOD and 29 ± 9% for C57BL/6 $CD4^+CD8^+$ immature thymocytes ($P = 0.37$, $n = 3$,). Moreover, this stimulus induced specific apoptosis in 44 ± 4% of NOD and 36 ± 9% of C57BL/6 $CD4^+CD8^-HSA^+$ semimature thymocytes ($P = 0.45$, $n = 4$). We do not believe that differences in methods for thymocyte subset enrichment (panning versus cell sorting) or mode of thymocyte stimulation (TCRβ versus CD3ε mAb) are responsible for the differences between our results and those reported earlier[2], because we obtained similar percentages of cell death induced by TCR-CD3 ligation when we treated C57BL/6 or NOD cells with TCRβ mAb (H57.5.2.1) and when C57BL/6 cells were isolated by panning (data not shown).

We also compared *in vivo* thymocyte deletion induced by TCR-CD3 stimulation between NOD and C57BL/6 mice. When NOD and C57BL/6 mice were injected with 20 or 200 g CD3ε antibody, total thymic cellularity and numbers of immature $CD4^+8^+$ thymocytes decreased to a similar extent in mice of both strains (**Supplementary Fig. 2**). Although the injection of 20 g CD3ε mAb resulted in slightly less-efficient deletion of semimature $CD4^+CD8^-HSA^+$ cells in NOD mice than in C57BL/6 mice, the difference was not statistically significant. Nearly complete deletion of semimature thymocytes occurred in both mouse strains after injection of 200 g CD3 mAb. We obtained similar results by injecting mice with TCRβ antibody, indicating that differences in the mode of thymocyte stimulation are unlikely to be responsible for the differences between our results and those reported earlier[1].

The death of autoreactive $CD4^+CD8^+$ thymocytes requires Bim[2,3] but occurs independently of Fas-, FADD- and caspase-8-transduced 'death receptor' signaling in general[4] and the Apaf-1-caspase-9 apoptosome[5]. Moreover, killing of semimature thymocytes induced by TCR-CD3 occurs by the same Bim-mediated mechanisms (A.V. *et al.*, manuscript submitted). Collectively, our results indicate that killing induced by TCR-CD3 ligation killing occurs efficiently in immature and semimature thymocytes of NOD mice, indicating that abnormalities in these apoptotic mechanisms are unlikely to be responsible for their autoimmune disease. The reasons for the differences between our results and the previously published report[1] are not known at present.

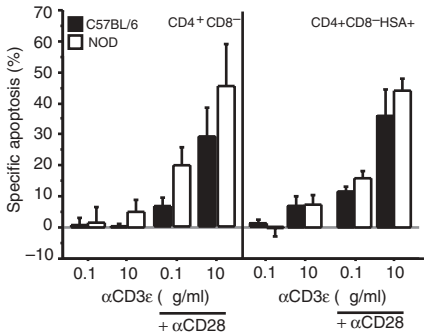

Figure 1 Immature $CD4^+CD8^+$ and semimature $CD4^+CD8^-HSA^+$ thymocytes from NOD mice are sensitive to apoptosis induced by TCR-CD3 ligation *in vitro*. Immature $CD4^+CD8^+$ and semimature $CD4^+CD8^-HSA^+$ thymocytes from NOD and C57BL/6 mice were purified by cell sorting and cultured for 20 h in plates coated with graded concentrations of CD3ε mAb (αCD3ε) in the presence or absence of optimal doses of CD28 antibodies (αCD28; methods, **Supplementary Fig. 1**). The amount of apoptosis induced specifically by TCR-CD3 ligation in semimature $CD4^+CD8^-HSA^-$ thymocytes from C57BL/6 and NOD mice was calculated by the following equation: (apoptosis induced by TCR-CD3 stimulation – spontaneous apoptosis) (100 – spontaneous apoptosis)%. Data represent arithmetic means ± s.e.m. of three to five independent experiments for animals of each genotype and treatment regime. We found no significant differences in the rates of specific killing in immature $CD4^+CD8^+$ or semimature $CD4^+CD8^-HSA^+$ thymocytes for C57BL/6- compared with NOD-derived cells ($P > 0.112$).

Note: Supplementary information is available on the Nature Immunology website

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