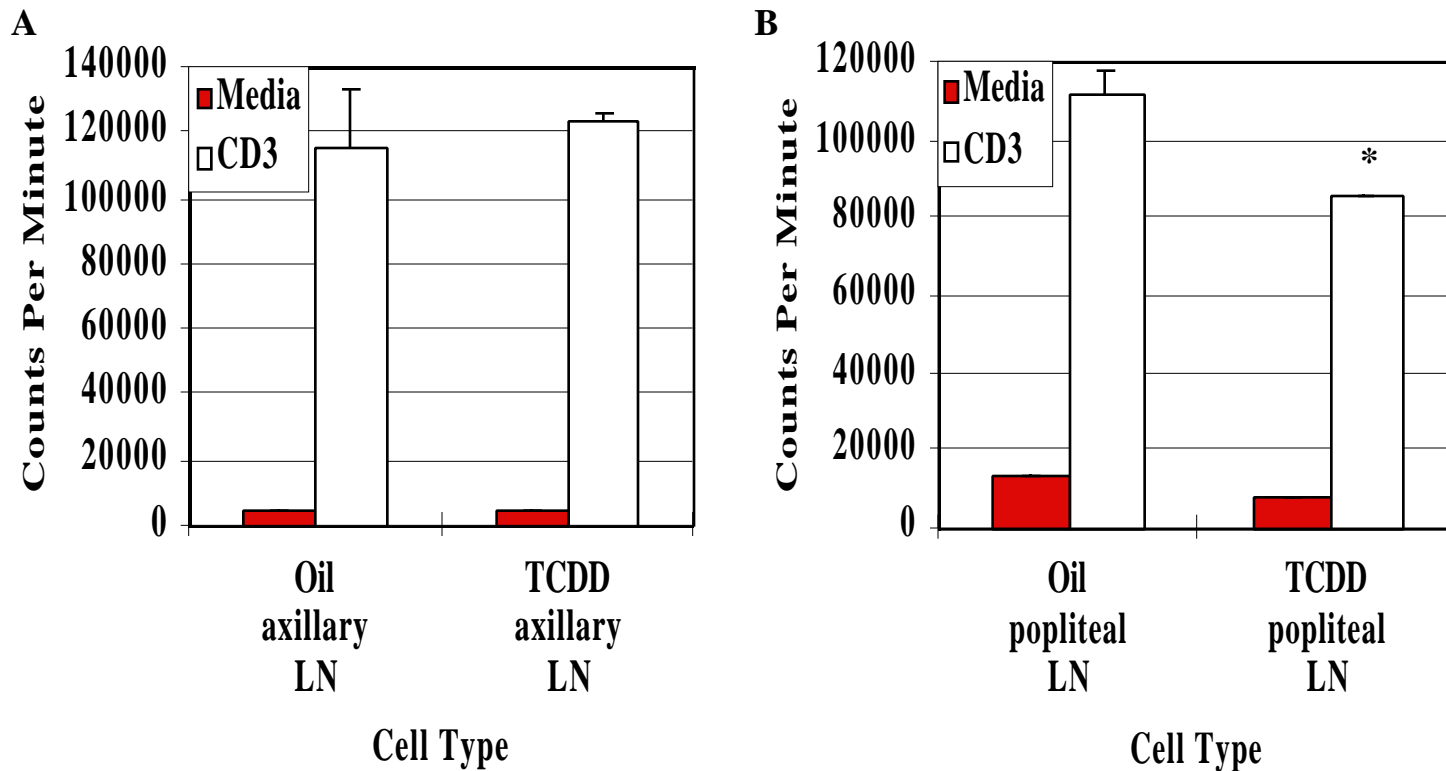
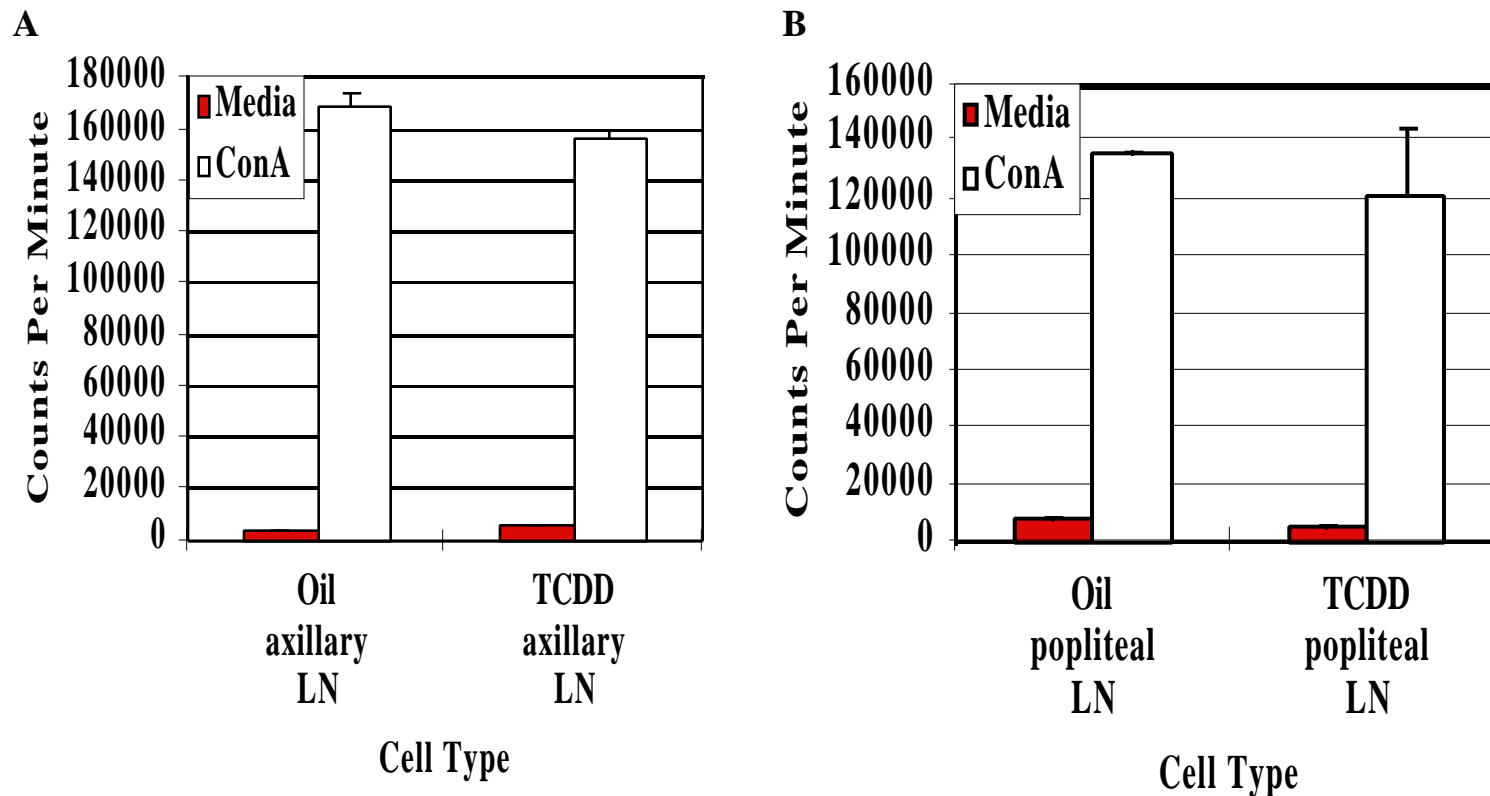


**Figure 2. Effect of TCDD on the responsiveness of axillary and popliteal LN cells to stimulation with IL-2.**

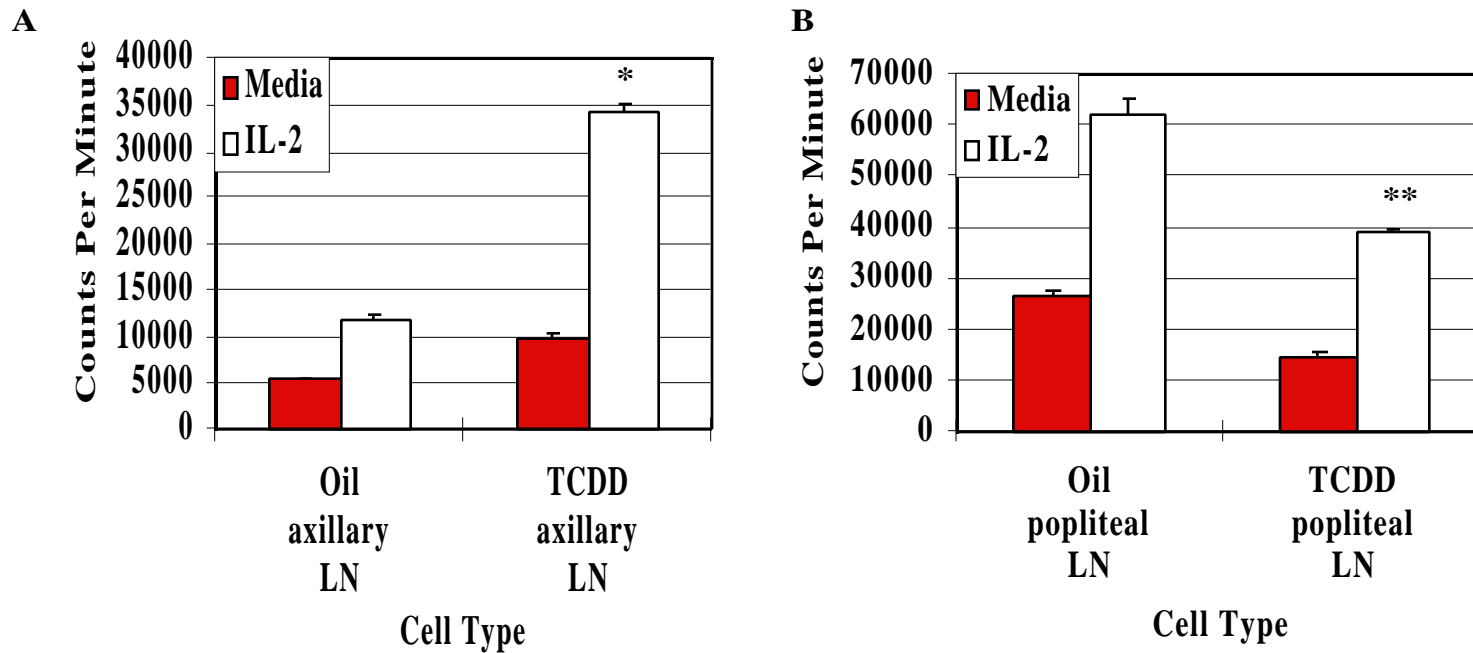
Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and with anti-CD3 mAbs into both rear footpads on day 2. Three days following treatment with vehicle/TCDD and one day following footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 48 hours with medium alone or in the presence of IL-2, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. No proliferative differences were observed between the resting LN cells from oil-treated and TCDD-treated animals (A). A small, but significant (\*\*) ( $p=0.005$ ), increase in proliferative rate was seen upon culturing activated lymph node cells from TCDD-treated animals with IL-2 (B).



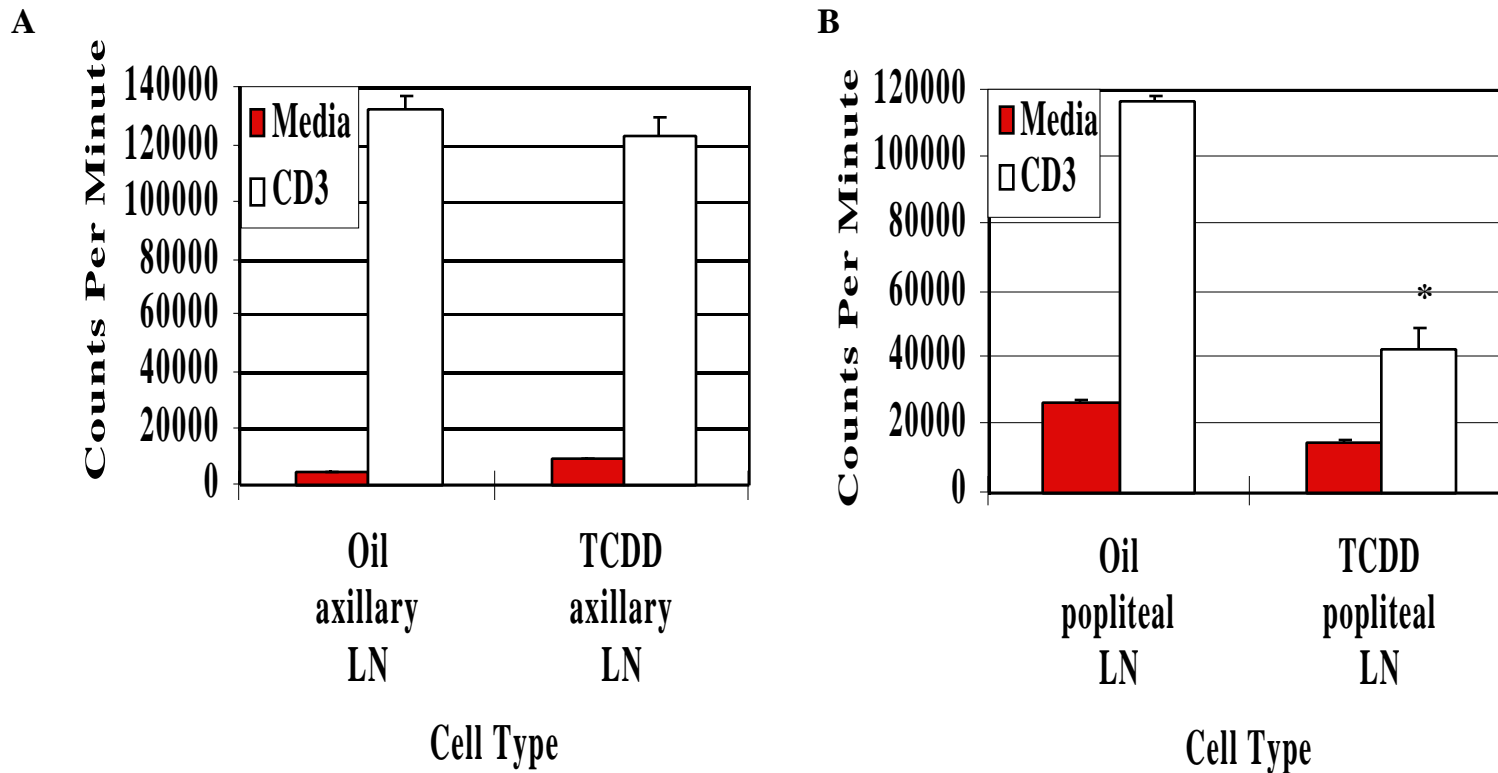
**Figure 3. TCDD-treatment decreases the responsiveness of popliteal, but not axillary LN cells to stimulation with anti-CD3 mAbs.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and with anti-CD3 mAbs into both rear footpads on day 2. Three days following treatment with vehicle/TCDD and one day following footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured with medium alone or in the presence of anti-CD3 mAbs for 48 hours, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean  $\text{cpm} \pm \text{SEM}$  of pooled triplicate cultures. Upon restimulation, an antigen-specific proliferative decrease was observed in the popliteal LN cells from TCDD-treated animals (\*) ( $p=0.01$ ) (B). No proliferative differences were observed in the resting lymph node cells (A).



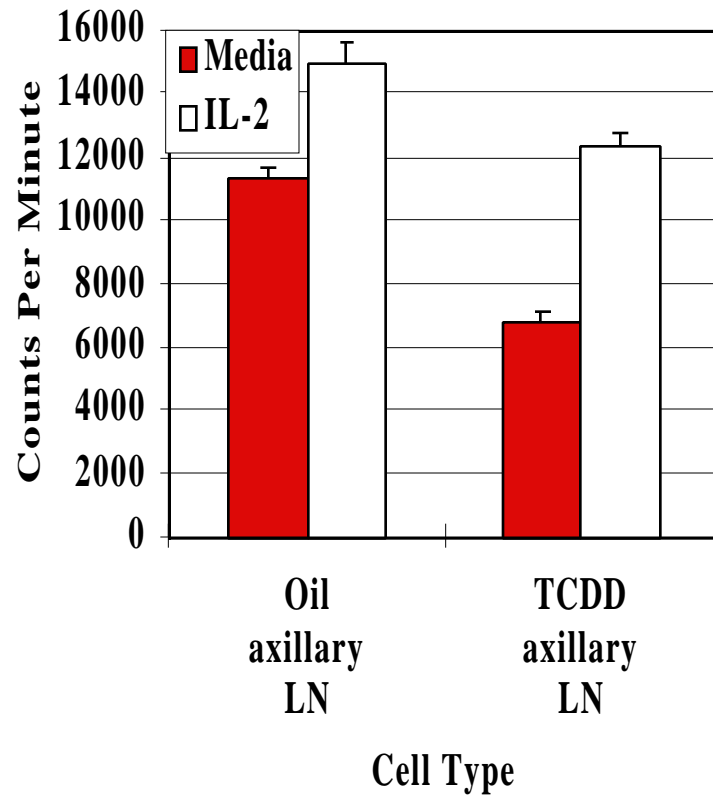
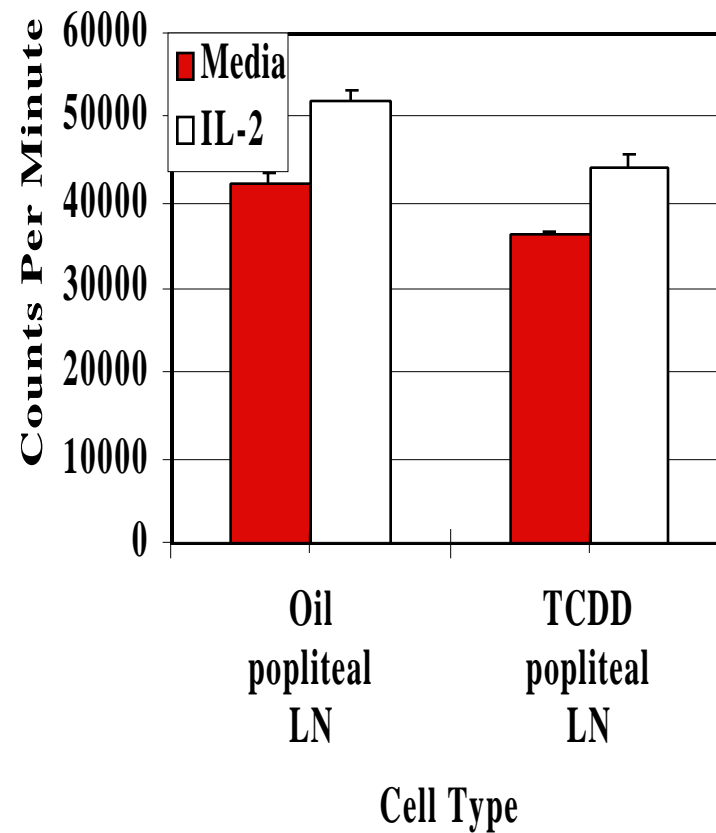
**Figure 4. Naive and activated T cells from TCDD-treated mice respond normally when stimulated in culture with the T cell mitogen ConA.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and with anti-CD3 mAbs into both rear footpads on day 2. Three days following treatment with vehicle/TCDD and one day following footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured with medium alone or in the presence of Concanavalin A for 48 hours, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. While an antigen-specific proliferative decrease was observed in the popliteal lymph node cells from TCDD-treated animals (Figure 3B), no proliferative decrease was observed upon culture with a non-specific T cell mitogen (B). As in previous studies, no proliferative differences were observed in the resting lymph node cell population.



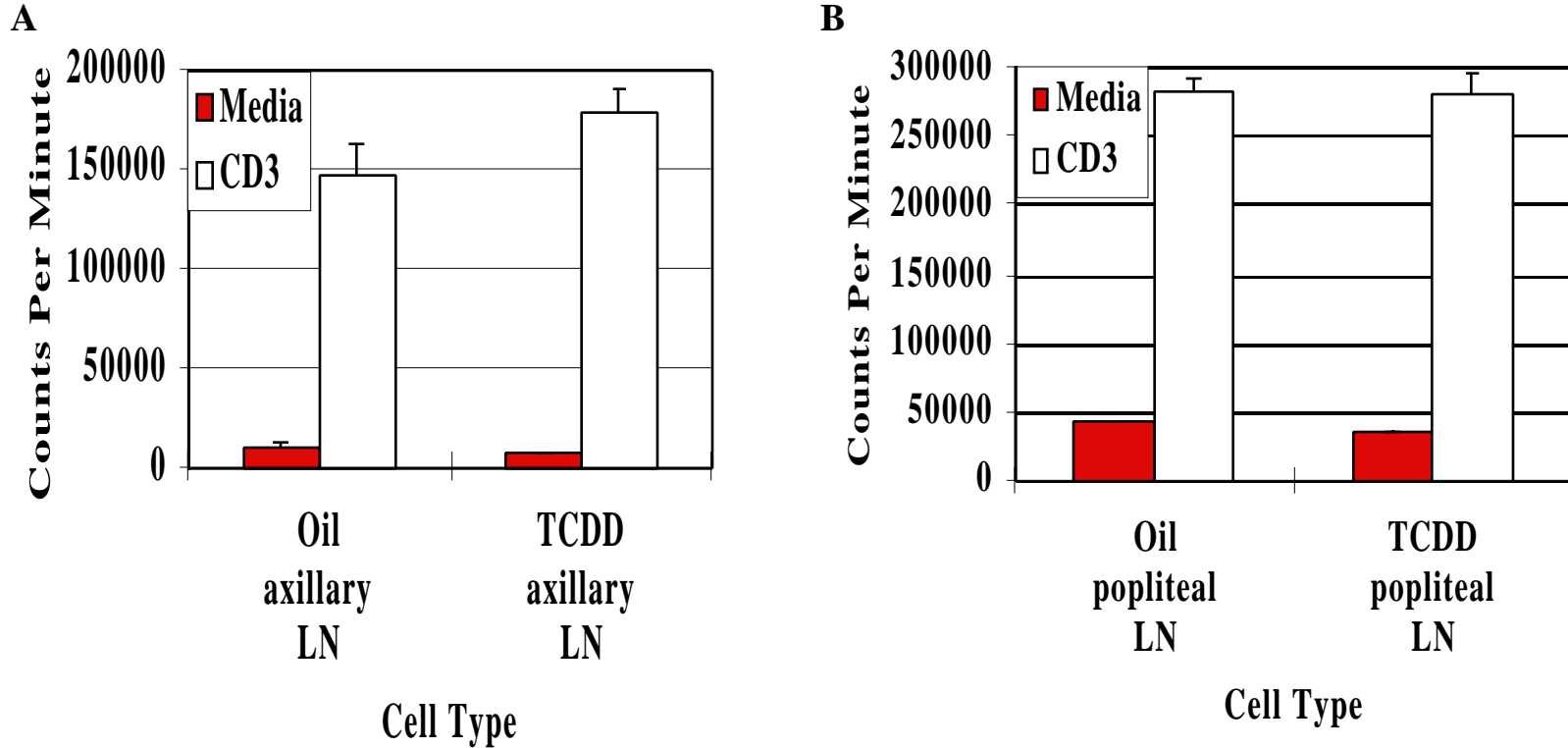
**Figure 5. Responsiveness of axillary and popliteal LN cells to stimulation with IL-2 *in vitro* one week after TCDD treatment.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and on the same day injected with anti-CD3 mAbs into both rear footpads. One week after vehicle/TCDD treatment and rear footpad immunization with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured with medium alone or in the presence of IL-2 for 24 hours, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. The resting lymph node cells from TCDD-treated animals showed an increased proliferative ability (\*) ( $p=0.0005$ ) over the control in response to culture with IL-2 (A). Activated lymph node cells from TCDD-treated animals, however, showed reduced proliferative ability (\*\*) ( $p=0.005$ ), compared to activated lymph node cells from oil-treated animals, when cultured with IL-2 (B).



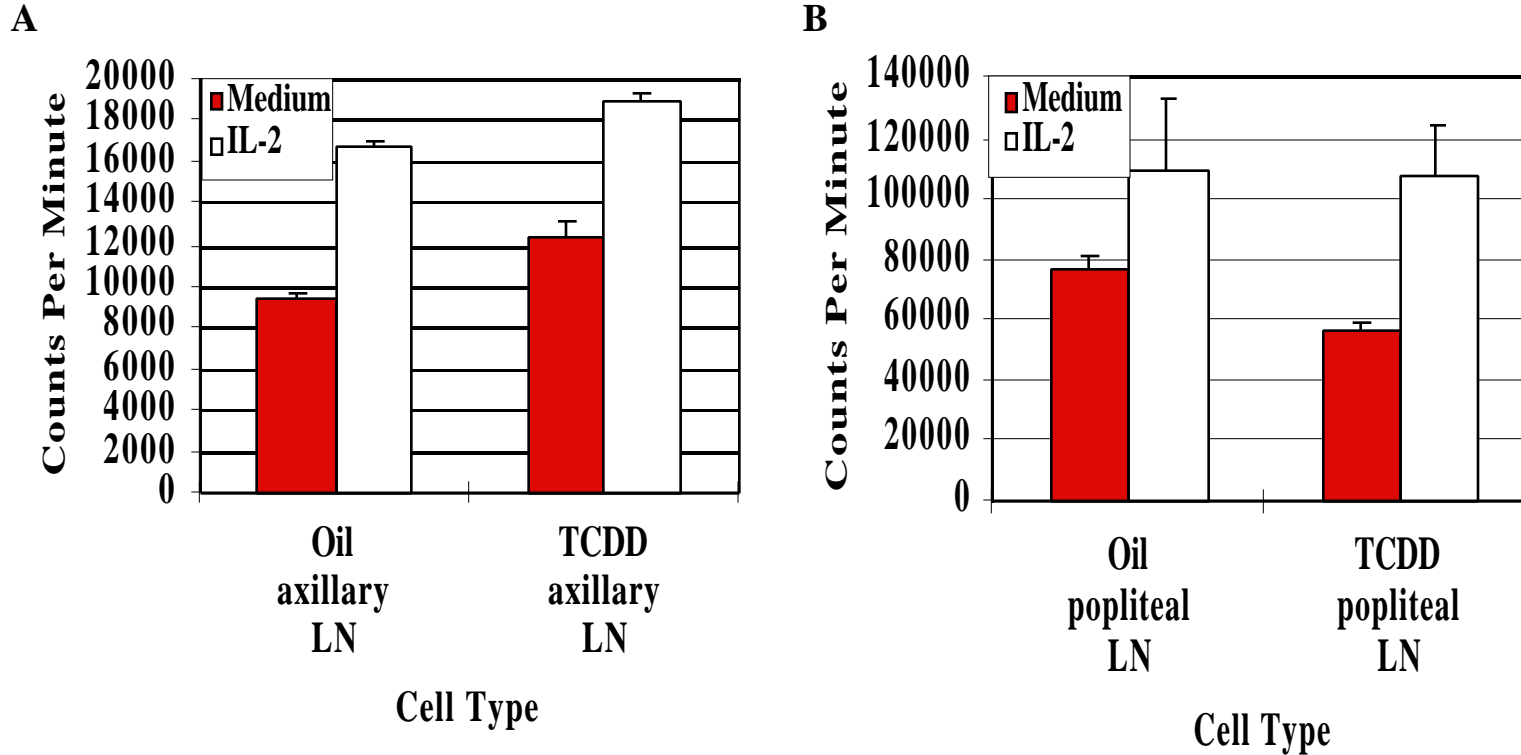
**Figure 6. TCDD decreases the responsiveness of activated, but not naive, T cells to stimulation with anti-CD3 mAbs one week after treatment.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and on the same day injected with anti-CD3 mAbs into both rear footpads. One week after vehicle/TCDD treatment and rear footpad immunization with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of anti-CD3 mAbs, and cellular proliferation was measured with a <sup>3</sup>H-thymidine uptake assay. Data are shown as mean cpm±SEM of pooled triplicate cultures. No proliferative differences were observed in the resting lymph node cell population (A). In the activated cell population from TCDD-treated animals, however, an antigen-specific proliferative decrease (\*) (p=0.0025) was clearly seen (B).

**A****B**

**Figure 7. Responsiveness of axillary and popliteal LN cells to IL-2 stimulation two weeks after TCDD treatment.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and on the same day injected with anti-CD3 mAbs into both rear footpads. Two weeks after vehicle/TCDD treatment and rear footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of IL-2, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. At this timepoint, the responsiveness of both the resting (A) and activated (B) lymphocytes from TCDD-treated animals remained similar to the proliferative levels of resting (A) and activated (B) lymphocytes from oil-treated mice.

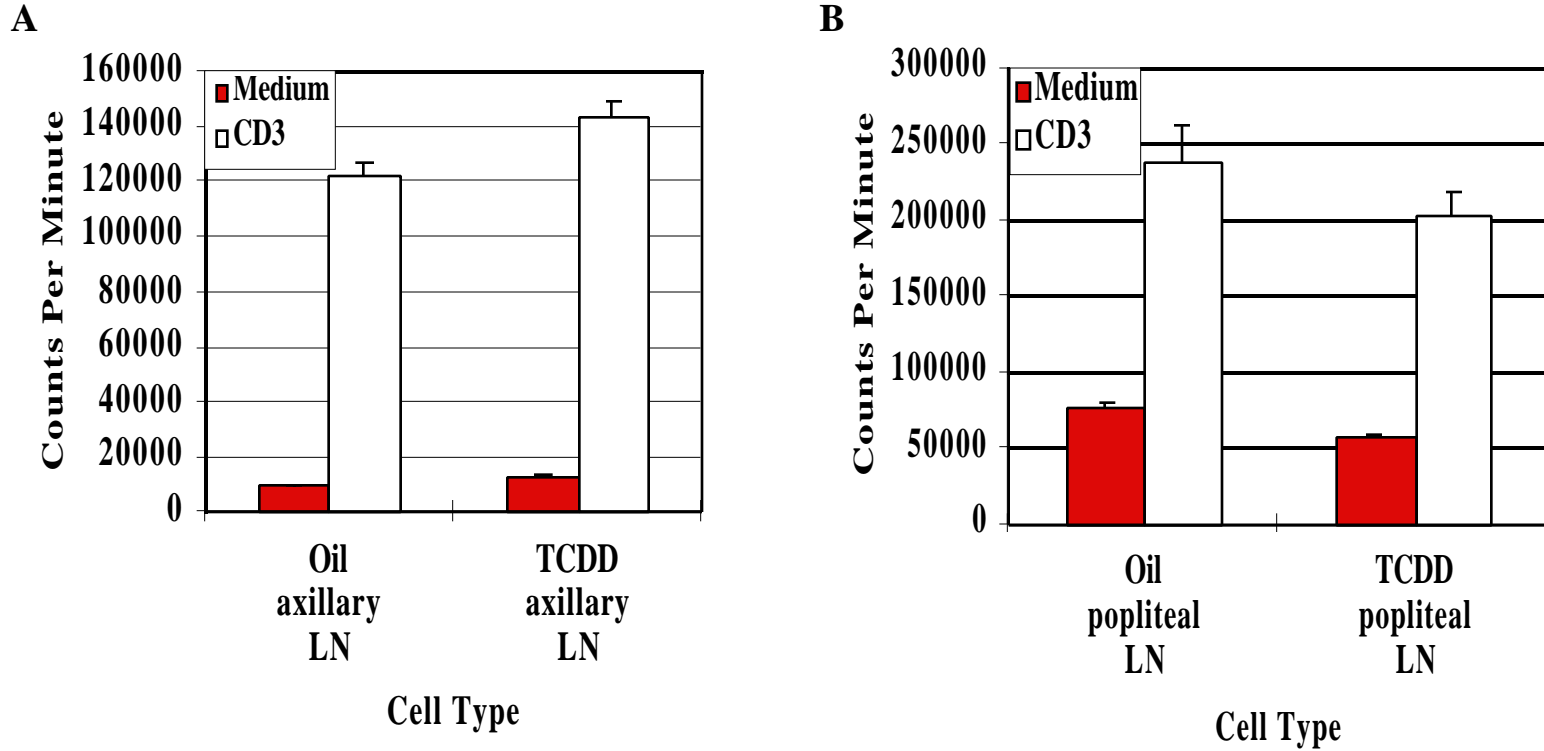


**Figure 8. Responsiveness of axillary and popliteal LN cells to anti-CD3 stimulation two weeks after TCDD treatment.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and on the same day injected with anti-CD3 mAbs into both rear footpads. Two weeks after vehicle/TCDD treatment and rear footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of anti-CD3 mAbs, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm  $\pm$  SEM of pooled triplicate cultures. At this timepoint, the responsiveness of both the resting (A) and activated (B) lymphocytes from TCDD-treated animals were similar to those of resting (A) and activated (B) lymphocytes from oil-treated animals.

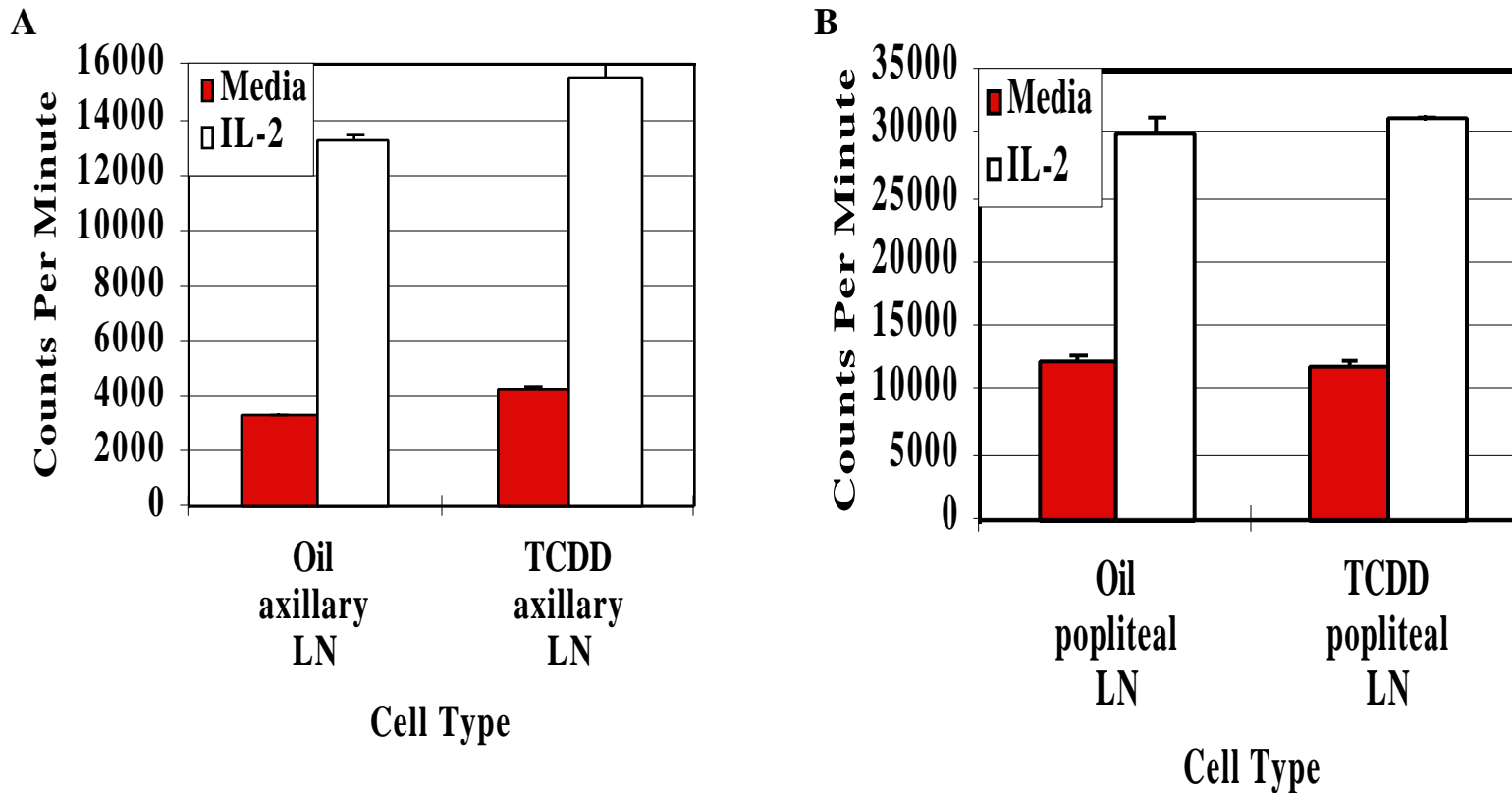


**Figure 9. Responsiveness of axillary and popliteal LN cells to IL-2 stimulation three weeks after TCDD treatment.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and on the same day injected with anti-CD3 mAbs into both rear footpads. Three weeks after vehicle/TCDD treatment and rear footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of IL-2, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. At this timepoint, the responsiveness of both the resting (A) and activated (B) lymphocytes from TCDD-treated animals remained similar to the proliferative responses of resting (A) and activated (B) cells from oil-treated mice.

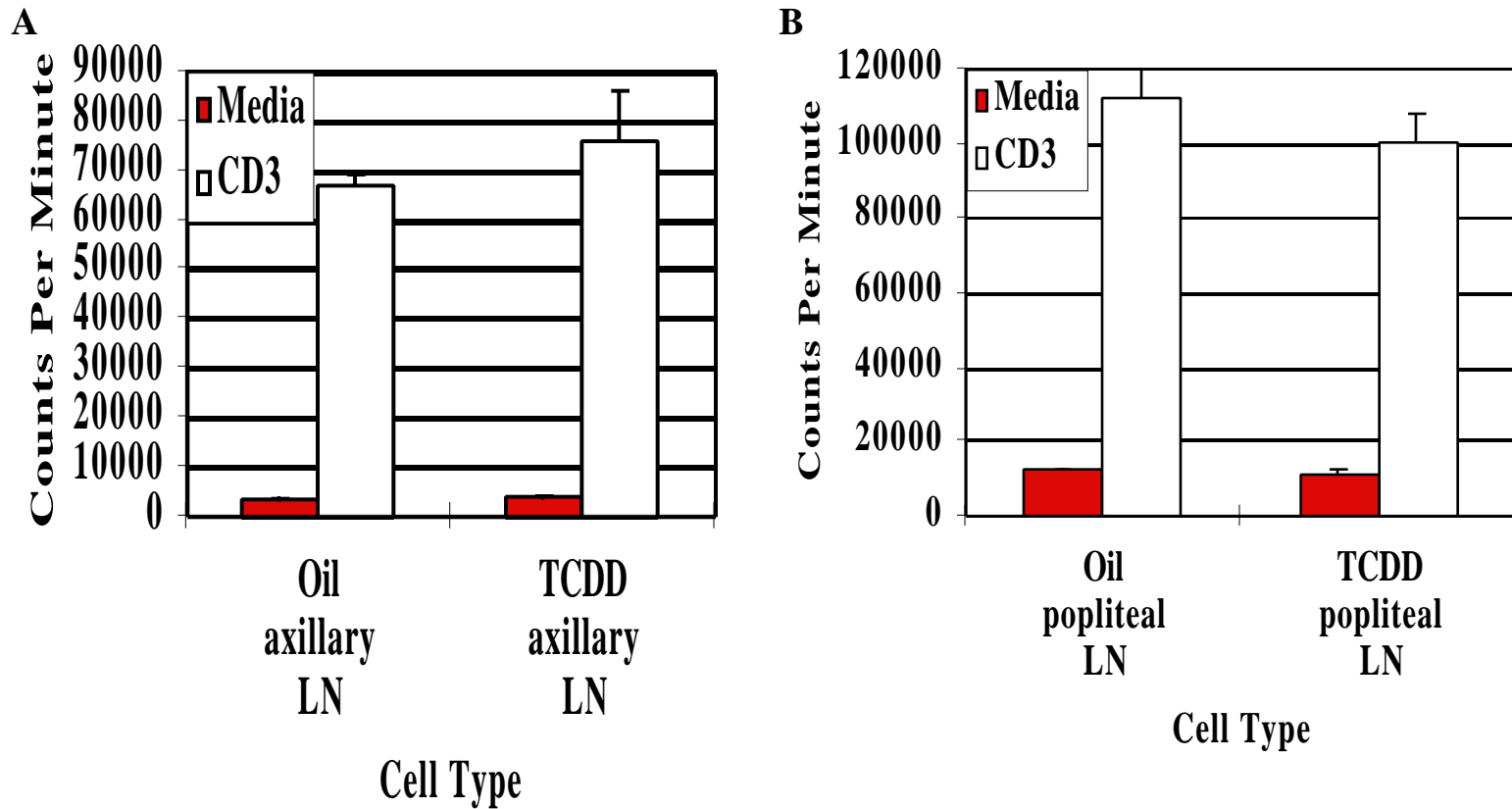




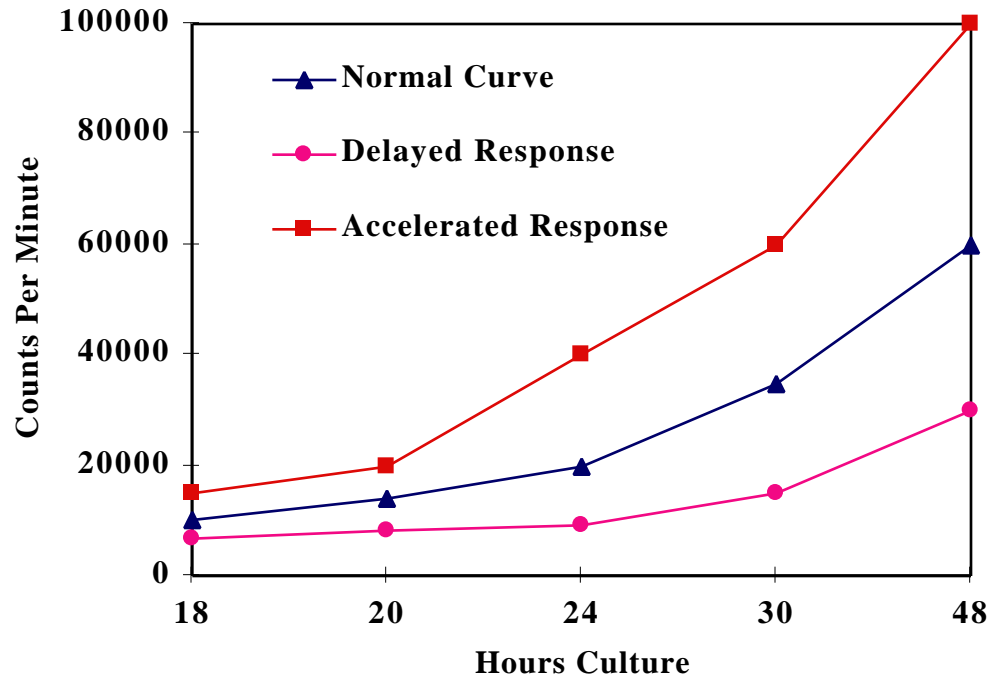
**Figure 10. Responsiveness of axillary and popliteal LN cells to anti-CD3 stimulation three weeks after TCDD treatment.** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0, and on the same day injected with anti-CD3 mAbs into both rear footpads. Three weeks after vehicle/TCDD treatment and rear footpad immunizations with anti-CD3 mAbs, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of anti-CD3 mAbs, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. At this timepoint, the responsiveness of both the resting (A) and activated (B) lymphocytes from TCDD-treated animals remained similar to the proliferative responses of resting (A) and activated (B) cells from oil-treated mice.



**Figure 11. *TCDD does not alter the responsiveness of resting popliteal LN T cells to stimulation with IL-2.*** Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0. One week after vehicle/TCDD treatment, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of IL-2, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. Axillary and popliteal lymph node cells respond similarly to culture with IL-2.

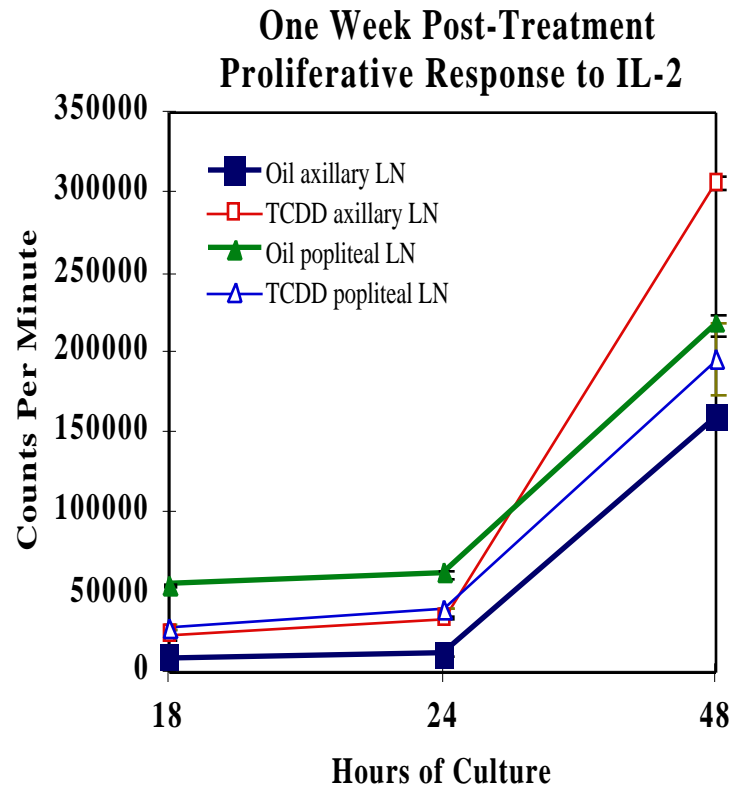


**Figure 12.** *TCDD does not alter the responsiveness of resting popliteal LN T cells to stimulation with anti-CD3 mAbs.* Groups of 5 C57BL/6 mice were injected with TCDD or the vehicle on day 0. One week after vehicle/TCDD treatment, axillary (A) and popliteal (B) lymph nodes were removed. Cells were cultured for 24 hours with medium alone or in the presence of anti-CD3 mAbs, and cellular proliferation was measured with a  $^3\text{H}$ -thymidine uptake assay. Data are shown as mean cpm $\pm$ SEM of pooled triplicate cultures. Axillary and popliteal lymph node cells respond similarly to culture with anti-CD3 mAbs.

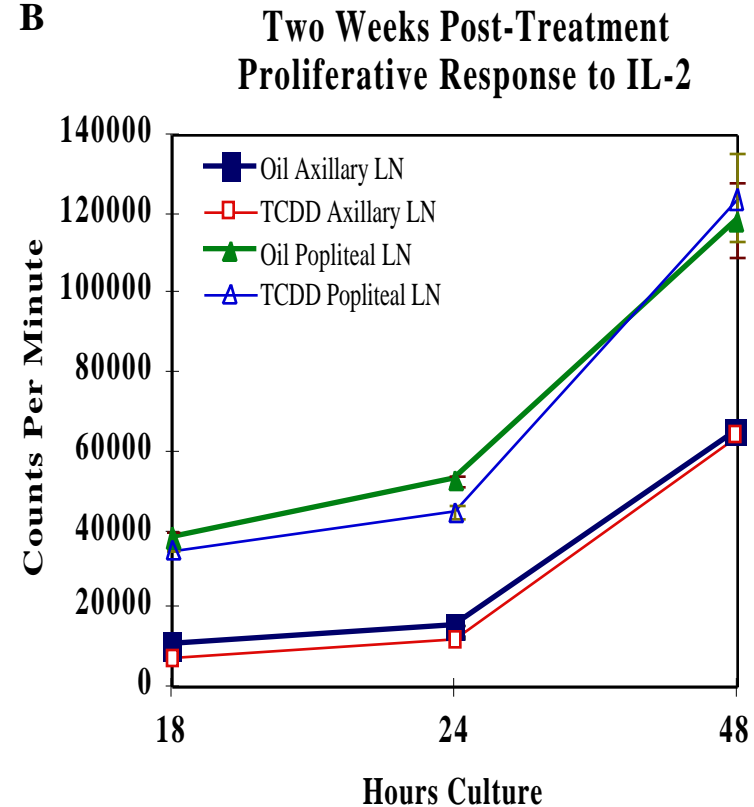


**Figure 13.** *A sample kinetics curve to illustrate accelerated and delayed immune responses.* An accelerated immune response shifts the curve up and to the left of the normal control, while a delayed immune response shifts the curve down and to the right of the normal control.

A

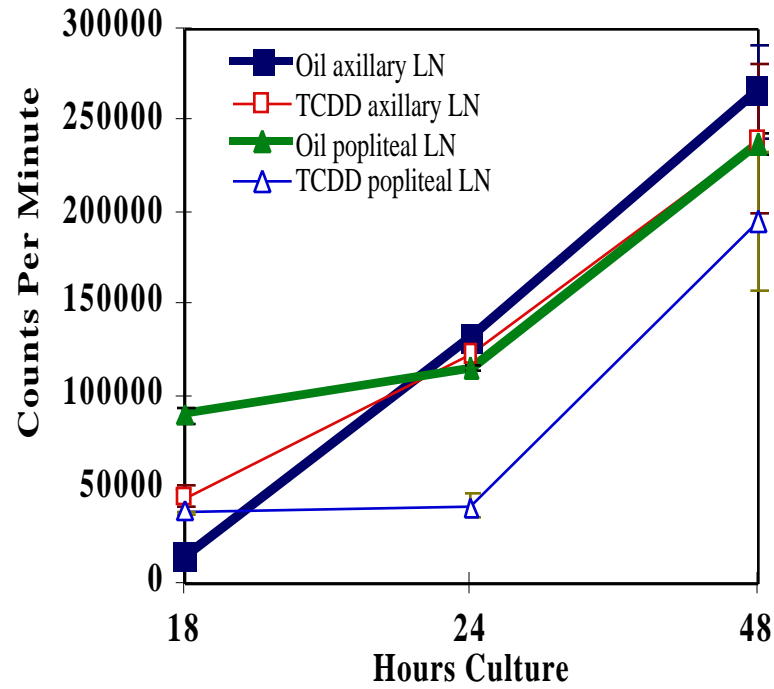


B

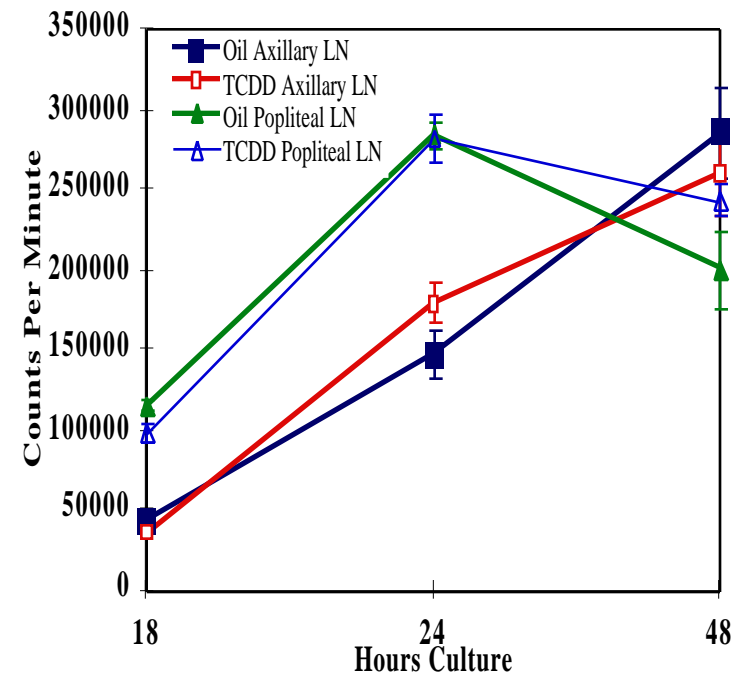


**Figure 14.** *Kinetics of the lymphocyte response to IL-2, one and two weeks following vehicle/TCDD treatment and footpad immunizations with anti-CD3 mAbs.* One (A) and two (B) weeks after vehicle/TCDD treatment and rear footpad immunizations with anti-CD3 mAbs, axillary and popliteal lymph nodes were removed. Cells were cultured for 18, 24, and 48 hours in the presence of IL-2. Proliferative rates were assessed by a  $^3\text{H}$ -thymidine uptake assay. Data are shown as  $\text{cpm} \pm \text{SEM}$ ;  $n=3$ . The SEM was often too small to extend beyond the identifying symbol.

**A** One Week Post-Treatment  
Proliferative Response to CD3



**B** Two Weeks Post-Treatment  
Proliferative Response to CD3



**Figure 15. Kinetics of the lymphocyte response to anti-CD3 mAbs, one and two weeks following vehicle/TCDD treatment and footpad immunizations with anti-CD3 mAbs.** One (A) and two (B) weeks after vehicle/TCDD treatment and rear footpad immunizations with anti-CD3 mAbs, axillary and popliteal lymph nodes were removed. Cells were cultured for 18, 24, and 48 hours in the presence of anti-CD3 mAbs. Proliferative rates were assessed by a  $^3\text{H}$ -thymidine uptake assay. Data are shown as  $\text{cpm} \pm \text{SEM}$ ;  $n=3$ . In A, the SEM was often too small to extend beyond the identifying symbol.