SUPPLEMENTAL FIGURE 1



Supplemental Figure 1. Anti-CD79b mAb does not effect T cells populations in vivo. DBA/1 mice (n=5 per group) were treated with 1.0 mg of anti-CD79b mAb (HM79) or hamster IgG (hIgG) as control 2 hours before immunization with CII. 7, 14 and, 21 days post immunization, mice were sacrificed and spleen were collected and processed as described in Methods. The percentage (A) and number (B) of T cells (CD3+) of splenocytes were assessed by flow cytometry.

SUPPLEMENTAL FIGURE 2



Supplemental Figure 2. B cell Depletion by anti-CD79b mAb is FcR and Complement Independent. A. Wild-type C57BL/6 or FcR γ -/- mice (n=3-4 per group) were injected with 0.5 mg of control polyclonal hamster IgG(hIgG), anti-CD79b mAb (HM79) or 0.25 mg of anti-CD20 mAb (18B12). Tissues were harvested for analysis one week later. The absolute number of (left panel) and percentage of splenic B cells (CD19+) (middle panel) and the percentage of peripheral blood B cells (CD19+) (right panel) were assessed. This experiment is representative of three independent experiments. B. Wild-type C57BL/6 or Fc γ RIIB-/- mice (n=3-4 per group) were injected with 0.5 mg of control polyclonal hamster IgG (hIgG) or anti-CD79b mAb (HM79). One week later, tissues were harvested for analysis. The absolute number of splenic B cells (CD19+) (left panel), the percentage of splenic B cells (middle panel) and the percentage of peripheral blood B cells (CD19+) (right panel) were assessed. This experiment is representative of two independent experiments. C. Wild-type C57BL/6 mice (n=3-4 per group) were treated with 12.5 U CVF every other day. On day 0, the mice were injected with 0.5 mg of control polyclonal hamster IgG (hIgG) or anti-CD79b mAb (HM79). Prior to injection with antibody, the concentration of plasma C3 was determined (left panel). One week after injection, tissues were harvested for analysis. The percentage of splenic B cells (CD19+) (middle panel) and peripheral blood B cells (CD19+) (was determined. B cell populations were discriminated as described in Methods. This experiment is representative of two independent experiment (left panel). One week after injection, tissues were harvested for analysis. The percentage of splenic B cells (CD19+) (middle panel) and peripheral blood B cells (CD19+) (was determined. B cell populations were discriminated as described in Methods. This experiment is representative of two independent experiments.

SUPPLEMENTAL FIGURE 3



Supplemental Figure 3. The D265A mutation does not affect anti-CD79b in binding to B cells or induction of B cell loss. Peak cells were transfected with a CD79b construct, harvested 48 hours later and incubated with the indicated Abs. The cells were then analyzed by FACS to determine the relative surface binding of isotype controls (open trace) and the specific antibodies (shaded trace); anti-CD79b (left panel), m-anti-CD79b (middle panel) or m-anti-CD79b D/A (right panel). B. Wild-type C57BL/6 mice were injected with 0.5 mg of control mouse IgG (mIgG), m-anti-CD79b mAb or m-anti-CD79b-D/A. Tissues were harvested for analysis one week later. The absolute number of splenocytes and the percentage of B cells (CD19+) among splenocytes by absolute number, and percentage. Data are representative of 2 independent experiments as described above and consist of 5-6 mice. C. The frequency of peripheral blood and lymph node B cells (CD19+). D. The frequency (left panel) and absolute numbers (right panel) of immature (CD93+) and mature (CD93-) B cells among the total splenic B cells (CD19+). E. The frequency (left panel) and absolute number (right panel) of follicular B cells (CD23+, CD21+) B cells among splenic B cells (CD19+). F. The frequency (left panel) and absolute number (right panel) of marginal zone (CD21+, CD1dhi) B cells among splenic B cells (CD19+).