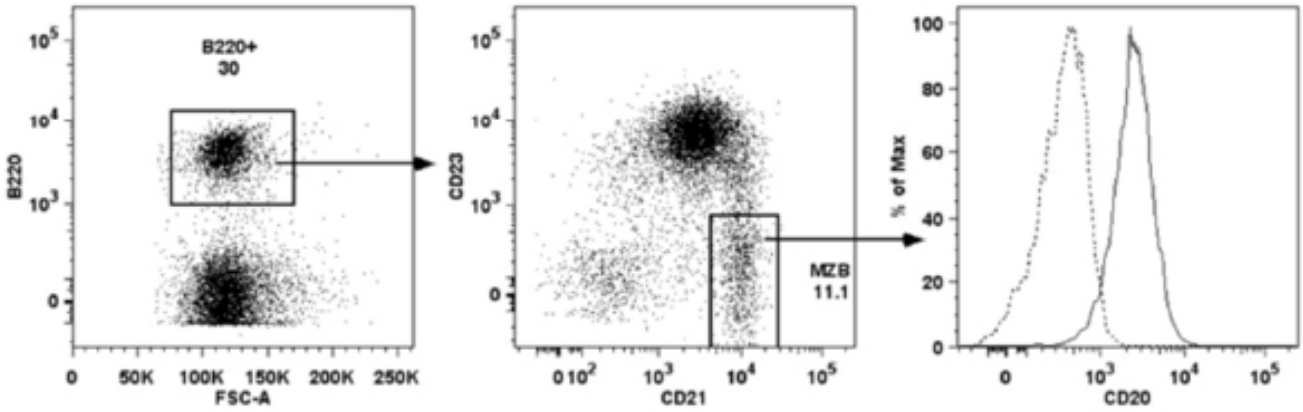
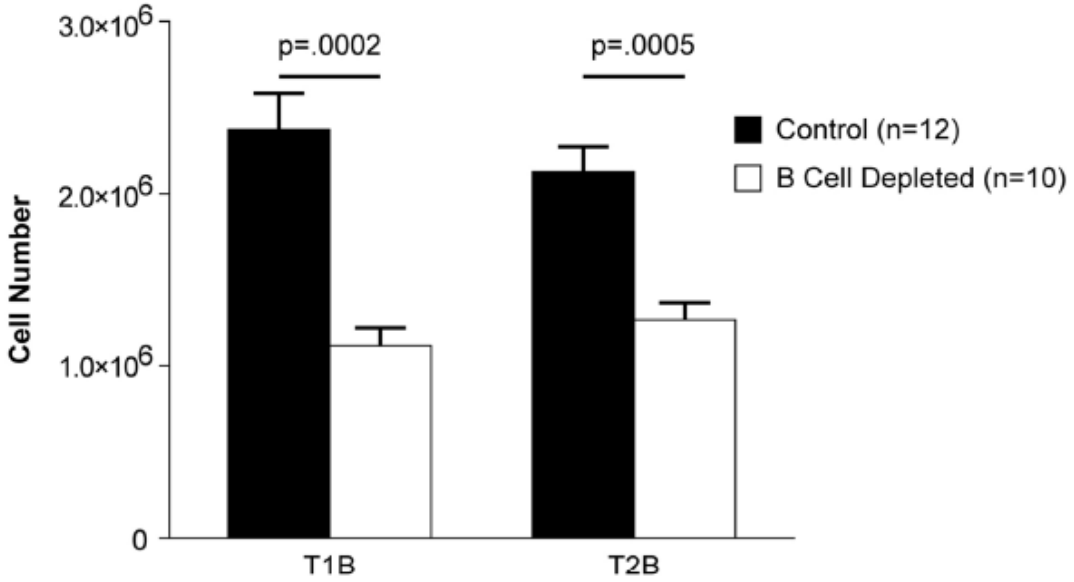
SUPPLEMENTARY DATA

Supplementary Figure 1. MZ B-lymphocytes in NOD mice express CD20. Staining of NOD splenic MZ B-lymphocytes gated on a B220+ CD21hi CD23- phenotype by an AP fluorochrome conjugated CD20-specific IgG2a 18B12 antibody is depicted by the solid histogram. Background staining of NOD MZ B-lymphocytes in the absence of the AP conjugated anti-CD20 antibody is depicted by the dashed histogram.

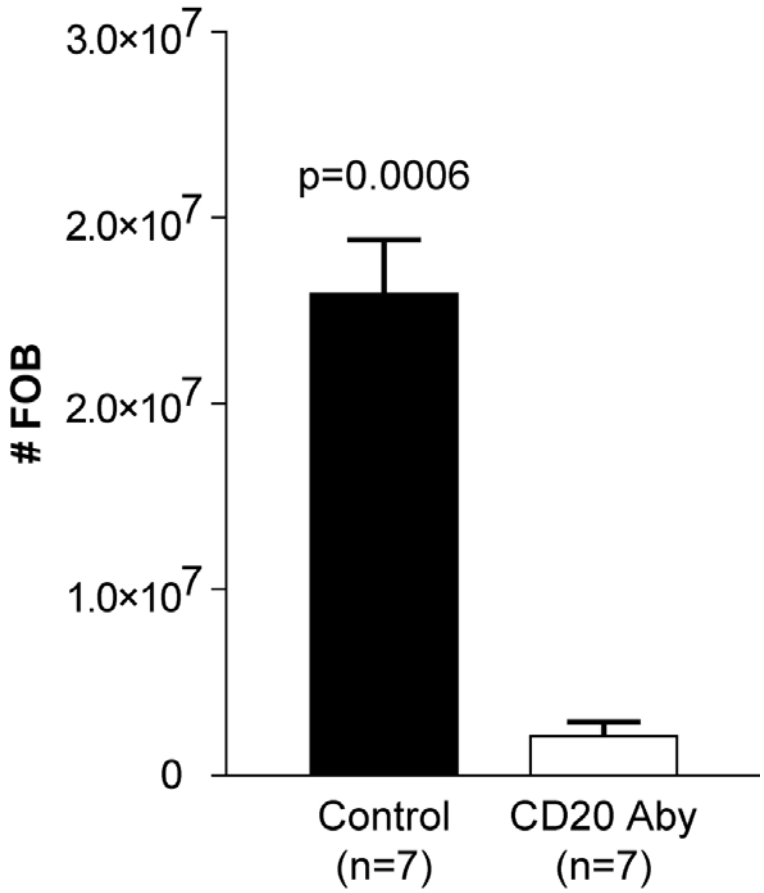


Supplementary Figure 2. Treatment of NOD mice with the CD20-specific IgG1 18B12 antibody depletes the immature splenic T1 (B220+ CD21- CD23-) and T2 (B220+ CD21hi CD23 int) subsets of B-lymphocytes. NOD female mice were treated with the CD20-specific or control antibody at 6-8 weeks of age and assessed four days later for various B-lymphocyte subsets.



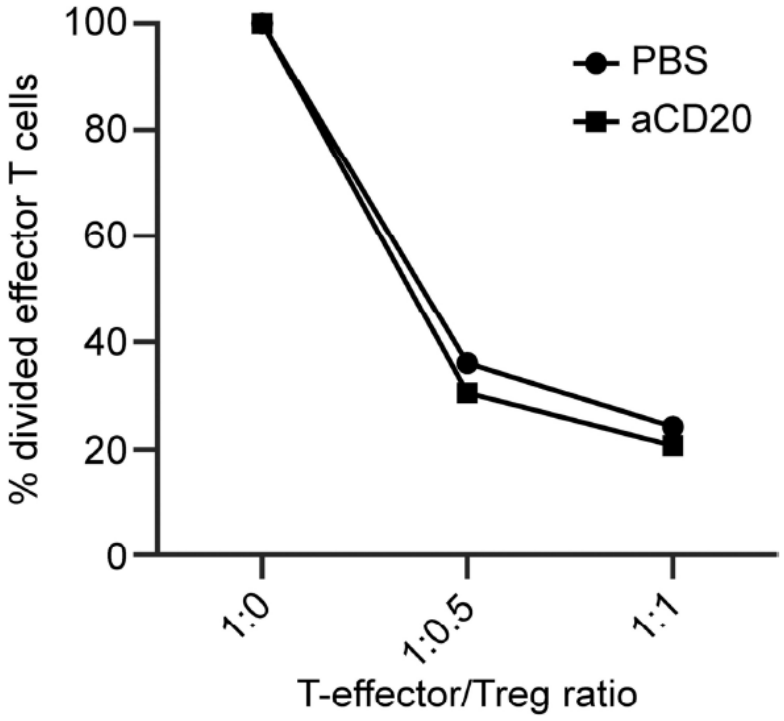
SUPPLEMENTARY DATA

Supplementary Figure 3. The inability of anti-CD20 treatment to block progression to diabetes when initiated in NOD mice that have already become IAA positive is not due to lost ability to mediate FO B-lymphocyte depletion. At diabetes onset splenic FO B-lymphocytes numbers were assessed in NOD females in which treatment with the CD20-specific or control antibody was initiated after IAA onset.



SUPPLEMENTARY DATA

Supplementary Figure 4. In vitro suppressive activity of Tregs from control and anti-CD20 treated NOD mice is equivalent on a per-cell basis. $CD4^+CD25^+$ Tregs were purified from NOD mice 4 days after receiving a second injection at 21-day intervals of control or CD20-specific antibody. Effector $CD4^+CD25^-$ T-cells were purified and labeled with CFSE. Labeled effector cells and Tregs were co-cultured in triplicate at the indicated ratios with 5µg/ml anti-CD3 (145-2C11) and NOD-scid splenocytes as a source of APC for 3 days. Proliferation of effector T-cells was determined by CSFE dilution. Data are normalized to the percentage of effectror CD4 T-cells proliferating in the absence of Tregs.



SUPPLEMENTARY DATA

Supplemental Figure 5. Joint anti-CD20/CD25 treatment of NOD mice numerically decreases Tregs, but not conventional CD4 and CD8 T-cells. NOD female mice at 8 weeks of age were co-injected with anti-CD20 and anti-CD25 or control antibody (n=3 per group). At 12 days post-treatment numbers of splenic (A) phenotypic Tregs (CD4+ FoxP3+ CD25+) and conventional (B) CD4 and (C) CD8 T-cells (both FoxP3-) were assessed by flow cytometry. Only levels of phenotypic Tregs were significantly less (p=X, ANOVA analyses) in anti-CD20/CD25 treated than control NOD mice.