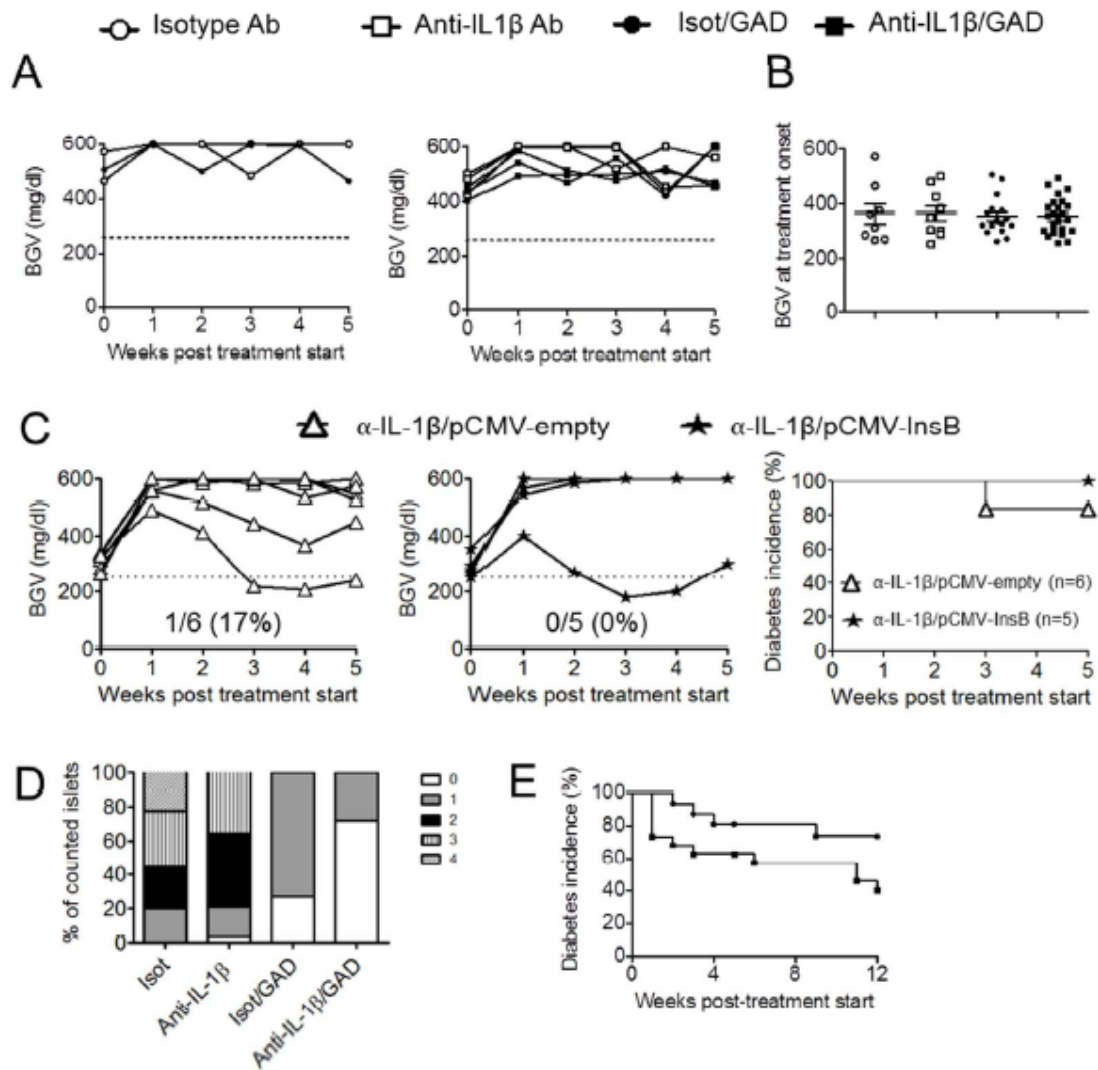


SUPPLEMENTARY DATA

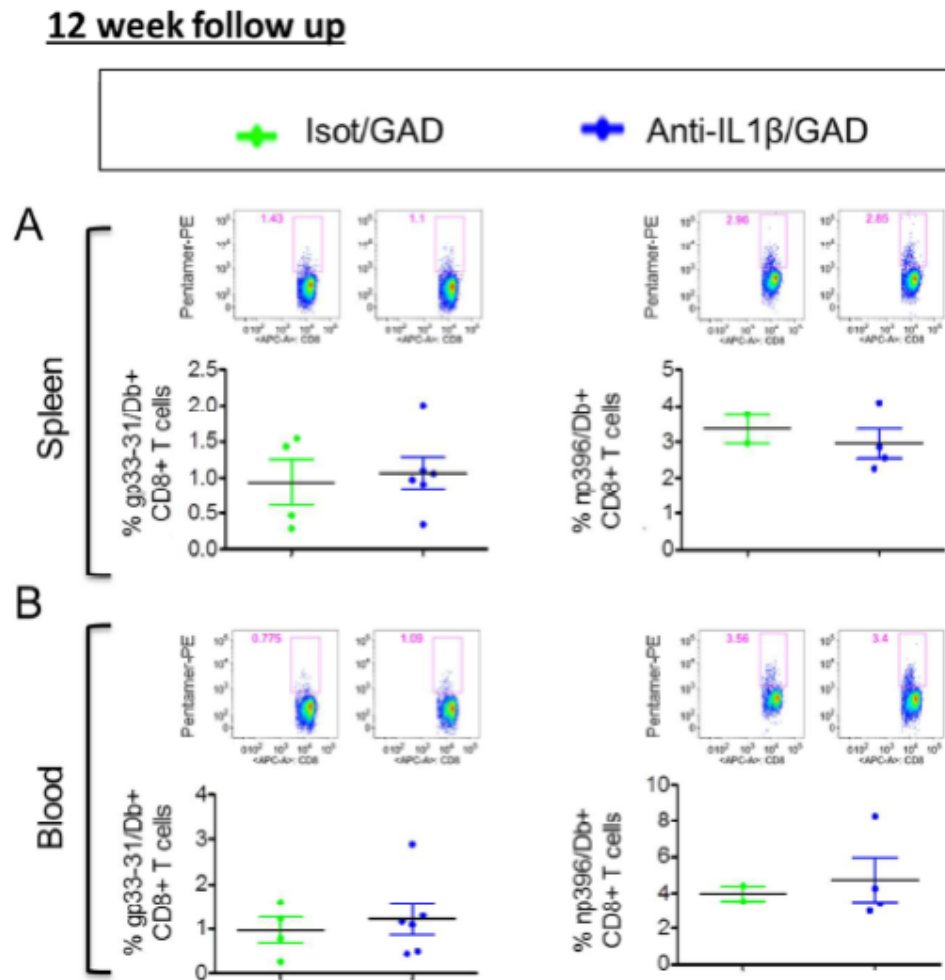
Supplementary Figure 1. (A) Individual BGV follow-up in groups of mice enrolled with BGV at onset between 400-573mg/dl. Data from one experiment with 1-3 mice per group. (B) Individual BGVs at treatment start. No significant different is observed. Mean \pm SEM for each group is represented. (C) Individual BGV follow-up and diabetes incidence in mice treated with anti-IL-1 β plus empty pCMV plasmid (empty triangles) or anti-IL-1 β (3 plus pCMV expressing islet autoantigen insulin B chain (closed stars). Data from two experiments with 1-3 mice per group. (D) Histology scoring upon hematoxylin and eosin staining confirming diabetes incidence curves after 5-week follow up : (0) no insulinitis, (1) peri-insulinitis with no islet destruction, (2) severe peri-insulinitis and some infiltrating insulinitis, (3) infiltrating insulinitis and some islet destruction, (4) infiltrating insulinitis and extensive islet destruction (or islet destroyed). Fractions were calculated based on observations from 11-26 islets and 2-3 mice per group. (E) Cumulative diabetes incidence after 12-week follow up in mice enrolled with BGV between 250-400mg/dl. This graph combines data from mice that euthanized both at 5 and 12 weeks after onset. P-value is 0.059.

5-week follow-up



SUPPLEMENTARY DATA

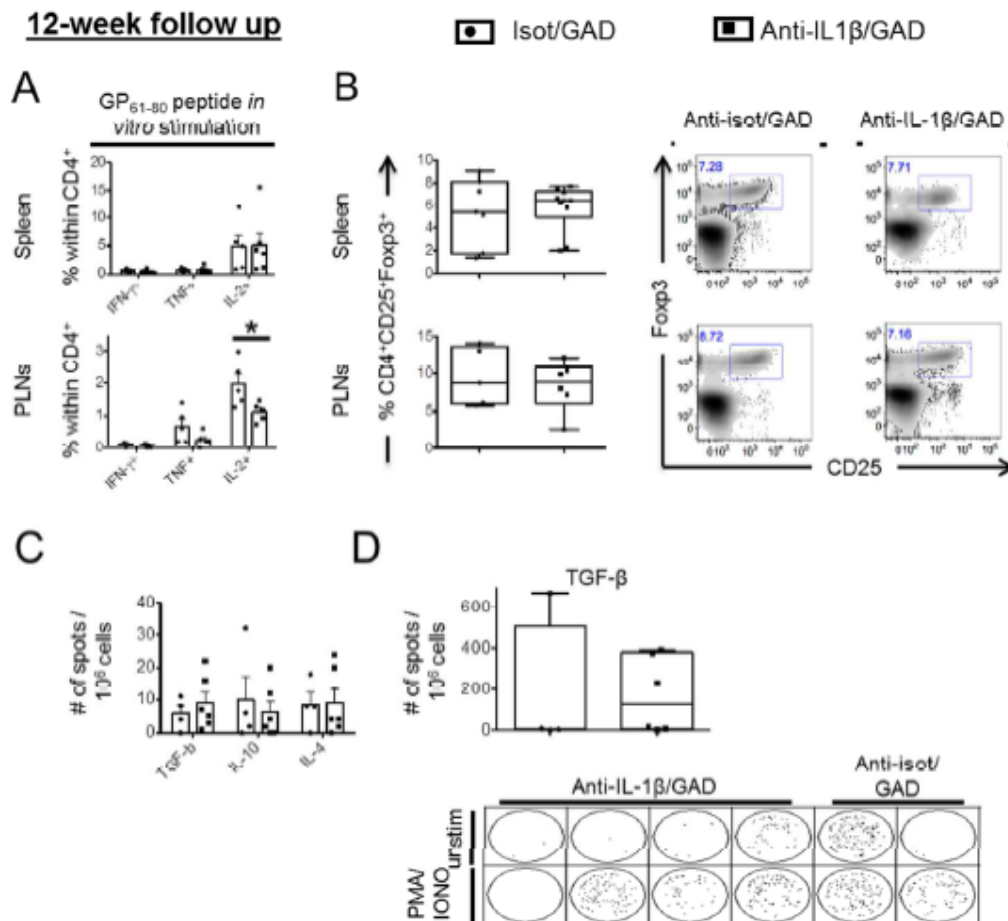
Supplementary Figure 2. Effector CD8 T-cell responses at 12 weeks post-onset are largely unaffected by the CT in spleen and blood. (A) Frequencies of gp33/Db (left panel) or np396/Db (right panel) pentamer- positive CD8 T cells in the spleen (A) or blood (B) of treated mice as well as representative FACS plots depicting frequencies of np396/Db pentamer-positive CD8 T cells. Cells were pre-gated on Live/dead Aqua-negative, and CD8+CD19- cells. Results from 2 pooled experiments with 2-4 mice per group are shown.



SUPPLEMENTARY DATA

Supplementary Figure 3. Effector and regulatory CD4 T-cell responses in the spleen and PLNs of isot/GAD- and CT-treated mice. (A) Frequencies of IFN- γ -, TNF- and IL-2-producing GP61-specific CD4⁺ effector T cells in spleen (upper panels) and PLNs (lower panels) of treated mice. After 12 weeks post-treatment, cells from spleens and PLNs were left unstimulated, or stimulated for 4 to 5 hours with 10 μ g/mL (CD4 T-cell specific) GP61 peptide in the presence of brefeldin A (10 μ g/mL). Data are expressed as % of gated CD4⁺ cells. (B) Frequencies of Foxp3-expressing CD4⁺CD25⁺ T regulatory cells in spleen (upper panels) and PLNs (lower panels) of treated mice. Data are expressed as % of gated CD25⁺Foxp3⁺ within CD4⁺ cells. (C,D) Cells isolated from spleen (C) and PLNs (D) were submitted to ELISpot assays for TGF- β , IL-10 or IL-4 as previously done by Sarikonda et al ([#]). Shown in (D) is an example of raw data for TGF- β ELISpot in PLN. The number of spots is calculated from duplicate wells where spots from unstimulated wells were subtracted from wells stimulated with PMA and ionomycin. Results from 2 or 3 pooled experiments with 2-6 mice per group are shown. *p<0.05.

[#] Ghanashyam Sarikonda, Sowbarnika Sachithanatham, Yulia Manenkova, Tinalyn Kupfer, Amanda Posgai, Clive Wasserfall, Philip Bernstein, Laura Straub, Philippe P. Pagini, Darius Schneider, Teresa Rodriguez Calvo, Marilynne Coulombe, Kevan Herold, Ronald G. Gill, Mark Atkinson, Gerald Nepom, Mario Ehlers, Teodora Staeva, Hideki Garren, Lawrence Steinman, Andrew C. Chan, Matthias von Herrath. Transient B-Cell Depletion with Anti-CD20 in Combination with Proinsulin DNA Vaccine or Oral Insulin: Immunologic Effects and Efficacy in NOD Mice. *PLoS One*. 2013;8(2):e54712. doi: 10.1371/journal.pone.0054712. Epub 2013 Feb 6.



SUPPLEMENTARY DATA

Supplementary Figure 4. Frequencies, chemokine receptor and costimulatory molecule expression in CD8⁺, CD4⁺ or CD11b⁺ cells in the blood upon 4 days of treatment. (A) Blood frequencies of CD4⁺, CD8⁺ and CD11b^{high} cells after 4 days in mice treated with isotype antibody (open circles), anti-IL-1 β antibody (open squares), isotype antibody plus GAD65 DNA vaccine (filled circles) or anti-IL-1 β antibody plus GAD65 DNA vaccine (filled squares). (B) Chemokine receptor CXCR3 expression levels in pre-gated CD4⁺, CD8⁺ and CD11b^{high} cells represented in (A). (C) Costimulatory molecule CD40, CD80 and CD86 expression levels in pre-gated CD11b^{high} cells represented in (A). Results from 3 pooled experiments with 2-6 mice per group are shown.

4-day follow-up

