Supporting Information

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SI Methods

Antibodies and Reagents. Hamster anti-mouse CD3 antibody (clone 145-2C11), anti-mouse CD28 antibody (clone 37.51), and rat anti-TGF-β were purchased from BIO X CELL, and control hamster IgG was purchased from Jackson ImmunoResearch Laboratories. GC was purchased from Avanti Polar Lipids. Anti-CD3 (clone 145-2C11) for in vitro stimulation and reagents for FACS staining were purchased from BD PharMingen: CD16/CD32 (FcBlock), FITC, phycoerythrin (PE) or allophycocyanin (APC)-conjugated anti-CD4 (L3T4), and PE-conjugated anti-CD25 (PC61). Affinity-purified biotinylated goat anti-LAP polyclonal antibody and Strep-Avidin APC were purchased from R&D Systems. 7-AAD for staining dead cells was purchased from Sigma–Aldrich.

Flow Cytometric Analysis. Lymphocytes derived from blood, MLN, or spleen were resuspended in FACS buffer (PBS containing 2% vol/vol BSA). Cells were first incubated with FcBlock to exclude the possibility of nonspecific interaction. For CD4 staining, cells were incubated with FITC- or PE-conjugated antibodies for 30 min on ice. For LAP staining, cells were first stained with biotinylated LAP-specific antibody and were then stained with streptavidin allophycocyanin (SAV APC). Cells were also stained with 7-AAD to exclude dead cells. NKT cells were identified by α-galactosyl ceramide-loaded tetramers [National Institutes of Health (NIH) tetramer core] according to the NIH protocol, and results were compared with the use of anti-CD3 and anti-NK1.1 antibodies. Analysis was performed on a FACScan flow cytometer (Becton Dickinson) and calculated with FlowJo software (Tree Star, Inc.). Cells sorted for adoptive transfer experiments were stained in a similar manner and sorted for $CD4^+LAP^-$ or $CD4^+LAP^+$ using a FACSVantage SE (BD Biosciences). The purity of each population was determined to be 98% by flow cytometric analysis.

Proliferation Assay. Spleen or MLN cells were cultured in triplicate wells (5×10^5 cells per well) in serum-free medium X-VIVO 20 (BioWhittaker) with 1 μg/mL soluble anti-CD3 antibody. Proliferation was measured by scintillation counting after pulsing with 1 μCi [^{3}H]thymidine per well (NEN) for the last 16 h of a 72-h incubation period. When purified T cells (10×10^4 cells per well) were checked for proliferation, they were stimulated with 1 μg/mL anti-CD3 in the presence of DCs (10×10^3 cells per well) for 72 h and the proliferative response was measured as outlined above.

Cytokine Assay by ELISA. For cytokine assays, splenocytes or MLN cells were cultured (10^6 cells per well) in serum-free medium X-VIVO 20 with 1 μg/mL soluble anti-CD3 antibody. Supernatants were collected after 40 h for IL-2, IL-4, IL-6, IL-10, IFN-γ, and IL-17, or after 72 h for TGF-β, and quantitative ELISA was performed using paired antibodies and recombinant cytokines obtained from PharMingen, according to the supplier's recommendations. When purified T cells (10×10^4 cells per well) were checked for cytokine secretion, they were stimulated with 1 μg/mL anti-CD3 in the presence of DCs (10×10^3 cells per well) for 40 or 72 h and the cytokine content in the supernatant was measured as outlined above. The tissue cytokine profile was determined for the gut, liver, and pancreas. Part of the organ was weighed and homogenized on ice with TISSUEMISER (Fisher Scientific) in buffer containing PBS/BSA/Tween and protein inhibitor tablets (Roche). For each 100 mg of tissue, 1 mL of buffer was used. The homogenized material was centrifuged at 4 °C for 10–15 min at 6,000 × *g*. Supernatants were analyzed by ELISA for levels of IL-2, IL-6, IL-10, IL-17, IFN-γ, and TGF-β. For IL-1β ELISA, supernatants from T-cell and adipocyte cocultures were harvested at 96 h. For measurement of bioactive TGF-β1, $CD11c^+$ DCs were positively selected from the pancreas by magnetic separation using anti-CD11C microbeads (Miltenyi Biotec). Purified DCs were cultured in the presence of 1 μg/mL FLT3 ligand (R&D Systems). Culture supernatants were collected at 72 h poststimulation and used in a TGF-β1 immunoassay with a TGF-β1-specific detection kit from Quantikine.

Quantitative RT-PCR. MLN $CD11C^+$ DCs were isolated by magnetic separation using anti-CD11C microbeads. Total RNA was isolated from cell pellets using the RNA easy Mini Kit (QIAGEN) and stored at −80 °C. First-strand cDNA synthesis was performed on 0.5–1 μg of total RNA for each RNA sample using Taqman RT reagents (Applied Biosystems). The cDNA was amplified using sequence-specific primers for IL-10, and TGF-β and real-time PCR mix (Applied Biosystems) in an ABI 7500 cycler. All values were expressed as fold increase or decrease relative to the expression of GAPDH.

Histology. The liver, pancreas, and muscle were removed from control or treated mice and placed in 4% vol/vol formalin followed by paraffin-embedding. Five sections were prepared from each organ. The tissues were stained with H&E, and liver sections were additionally stained with oil-red-O. All sections were blindly scored by pathologists at the Brigham and Women's Hospital.

Adoptive Transfer. Recipient mice were adoptively transferred i.v. with 4×10^4 $CD4^+/LAP^+$ or $CD4^+/LAP^-$ cells sorted from spleens of donor mice fed with anti-CD3 + GC.

Statistical Analysis. Statistical significance was assessed by the two-tailed Student's *t* test. When there were more than two groups compared, differences were analyzed using one-way ANOVA. *P* values <0.05 were considered significant.

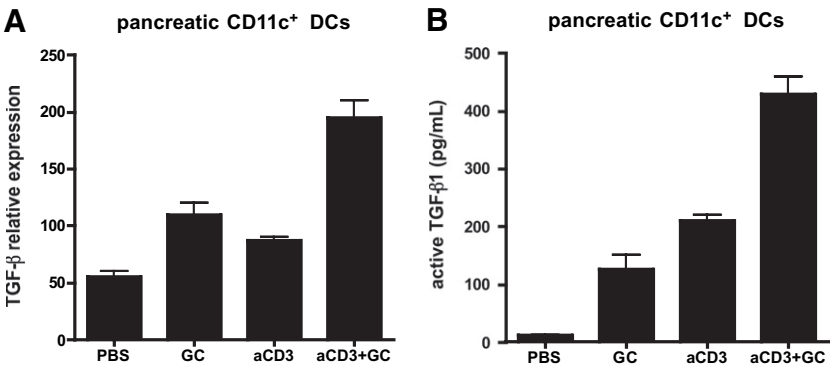

Fig. S1. TGF-β expression and production by CD11c$^+$ DCs. (*A*) CD11C$^+$ DCs were isolated from the pancreas 10 days after feeding using anti (a)-CD11c magnetic microbeads, and TGF-β mRNA expression was measured by quantitative PCR. Values are expressed as fold increase or decrease relative to the expression of GAPDH. (*B*) Pancreatic CD11c$^+$ DCs were stimulated in vitro with FLT3 ligand (1 μg/mL) for 72 h. Culture supernatants were used in ELISA for detection of bioactive TGF-β1.

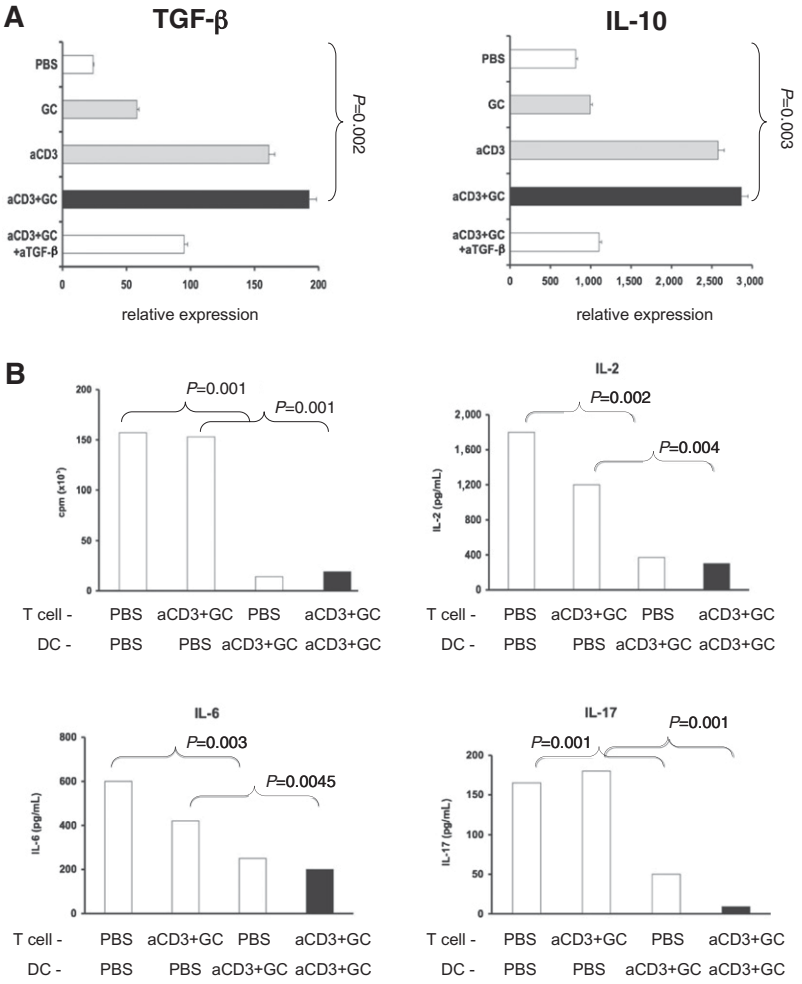

Fig. S2. DCs from the MLN of ob/ob mice fed anti (a)-CD3 + GC have increased expression of TGF-β and IL-10 and suppress proliferation and IL-2, IL-6, and IL-17 secretion. (*A*) CD11C$^+$ DCs were isolated from MLN 3 days after feeding using anti-CD11c magnetic microbeads, and TGF-β and IL-10 expression was measured by quantitative RT-PCR. Values are expressed as fold increase or decrease relative to the expression of GAPDH. (*B*) DCs isolated from the MLN of ob/ob mice (five per group) fed PBS or anti-CD3 + GC were tested for their ability to induce proliferation and secretion of IL-2, IL-6, or IL-17 by CD4 T cells harvested from PBS- or anti-CD3 + GC-fed mice. T cells were stimulated with 1 μg/mL anti-CD3 in vitro. Experiments shown in *A* were repeated three times with the same results. Error bars represent SD. Coculture experiments shown in *B* were repeated twice with the same results.

Table S1. Oral anti-CD3 + GC decreases glucose and AST in ob/ob mice

	PBS	GC	Anti-CD3	Anti-CD3 + GC
Glucose, mg/dL	367 ± 62	337 ± 33	316 ± 48	230 ± 36*
AST, U/L	416 ± 58	310 ± 53†	296 ± 44†	267 ± 41‡

Ob/ob mice (five per group) were fed daily with 5 μg of anti-CD3 plus 100 μg of GC daily for 5 days, and blood glucose, liver enzymes, and lipid levels were measured 10 days after feeding.

*$P < 0.001$ vs. PBS, GC alone, and anti-CD3 alone.

†$P < 0.005$ vs. PBS.

‡$P < 0.004$ vs. PBS.