Intestinal type 1 regulatory T cells migrate to periphery to suppress diabetogenic T cells and prevent diabetes development

Hua Yu1, Nicola Gagliani1, Harumichi Ishigame2, Samuel Huber3,4, Shu Zhu5, Enric Esplugues6, Kevan C. Herold3,4, Li Wenb, and Richard A. Flavell7,8,9

1Department of Immunobiology, Yale University, New Haven, CT 06520; 2Department of Internal Medicine, Yale University, New Haven, CT 06520; and 3Howard Hughes Medical Institute, Yale University, New Haven, CT 06520

Contributed by Richard A. Flavell, August 14, 2017 (sent for review April 5, 2017; reviewed by Katie Haskins and Matthias Von Herrath)

Growing insight into the pathogenesis of autoimmune diseases and numerous studies in preclinical models highlights the potential of regulatory T cells to restore tolerance. By using non-obese diabetic (NOD) BDC2.5 TCR-transgenic (Tg), and IL-10 and Foxp3 double-reporter mice, we demonstrate that alteration of gut microbiota during cohousing experiments or treatment with anti-CD3 mAb significantly increases intestinal IL-10-producing type 1 regulatory T (Tr1) cells and decrease diabetes incidence. These intestinal antigen-specific Tr1 cells have the ability to migrate to the periphery via a variety of chemokine receptors such as CCR4, CCR5, and CCR7 and to suppress proliferation of Th1 cells in the pancreas. The ability of Tr1 cells to cure diabetes in NOD mice required IL-10 signaling, as Tr1 cells could not suppress CD4+ T cells with a dominant-negative IL-10R. Taken together, our data show a key role of intestinal Tr1 cells in the control of effector T cells and development of diabetes. Therefore, modulating gut-associated lymphoid tissue to boost Tr1 cells may be important in type 1 diabetes management.

Significance
Past and current treatments for type 1 diabetes (T1D) have all suffered from adverse effects due to severe immune suppression or a lack of efficacy. A better understanding of the mechanisms and limitations of these therapies may help to maximize patient responses to treatment or elicit new ideas for a cure. Here, we discover a role for intestinal type 1 regulatory T (Tr1) cells in preventing autoimmune diabetes and provide mechanistic insight to explain the better efficacy of combination therapy in disease treatment. Our results also highlight the influence of dysbiotic gut microbiota on promotion of intestinal Tr1 cells and suggest that strategies targeting mucosal tissue to induce Tr1 in vivo might be used as a therapeutic approach for T1D.
not fully understood. In comparison with other animal housing conditions, our non-obese diabetic (NOD) mouse colony has a much lower diabetes incidence. As our mouse room also houses large numbers of mice lacking inflammasome components or effectors, such as Asc<sup>−/−</sup>, Nlrp3<sup>−/−</sup>, and Il18<sup>−/−</sup>, and these inflammasome-deficient mice harbor an altered intestinal microbiota that affect the disease susceptibility (26), we hypothesized that exposure to some of these dysbiotic microbiota may have an effect on the immune system and prevent diabetes development. Furthermore, microbiota have been shown to be capable of modulating the development of Th17 (27) and Treg cells (28); whether this beneficial outcome seen in our animal facility is due to the induction of intestinal Tr1 cells remains to be explored.

Here, we show that after alteration of gut microbiota through cohousing or administration of anti-CD3, the number of intestinal Tr1 cells was significantly increased. Up-regulation of IL-27 and TGF-β in the small intestine may account for the increased T cell differentiation. These regulatory T cells are mobile and have the ability to migrate to the periphery and sites of inflammation via different chemokine receptors, such as CCR4, CCR5, and CCR7. We also demonstrated that Tr1 cells could directly suppress diabetogenic T cells via IL-10 signaling and significantly delay disease development. Therefore, strategies targeting mucosal tissue to induce Tr1 cells in vivo might be used as a therapeutic approach to prevent diabetes or treat people with newly diagnosed T1D.

**Results**

**Colonization with Dysbiotic Gut Microbiota Promotes Intestinal IL-10–Producing CD4<sup>+</sup> T Cells and Protects NOD Mice from T1D.** To investigate whether the lower diabetes incidence in our animal room where NOD mice were housed somehow reflects colonization of gut flora from dysbiotic inflammasome-deficient mice, we cohoused female NOD mice purchased from the Jackson Laboratory at 3 wk of age with dysbiotic mice from our facility and monitored disease development. There was a clear difference in the cumulative disease incidence between the mice held under the two housing conditions. While 78% of noncohoused NOD mice developed diabetes by 25 wk of age, only 37% of cohoused NOD mice developed the disease (Fig. 1A). Concomitantly, intestinal IL-10–producing CD4<sup>+</sup> T cells were also significantly induced in cohoused Foxp3<sup>eGFP</sup> and IL-10<sup>CAGFP</sup> double-reporter NOD mice (Fig. 1B and C). Although the percentage of intestinal CD4<sup>+</sup>Foxp3<sup>+</sup> T cells decreased, the total number was not significantly changed (Fig. 1B and C). These results suggested that the protective function of dysbiotic gut microbiota might be attributed to the elevation of IL-10–producing CD4<sup>+</sup> T cells.

**Tr1 Cells Are Generated in the Small Intestine in Response to Anti-CD3.** To determine whether intestinal IL-10<sup>+</sup> CD4<sup>+</sup> T cells are generated in situ or migrate from the periphery, we used the anti-CD3–induced immune tolerance mouse model. From our past experience, administration of anti-CD3 leads to a strong induction of IL-10–producing cells in the gut. In accordance with previous findings (19), the frequency of Tr1 cells (CD4<sup>+</sup>Foxp3<sup>+</sup>IL-10<sup>+</sup>) was elevated in all examined organs, with the highest level seen in the intestine. By contrast, the frequencies of CD4<sup>+</sup>Foxp3<sup>+</sup>IL-10<sup>+</sup> and CD4<sup>+</sup>Foxp3<sup>+</sup>IL-10<sup>+</sup> T cells were not significantly changed by this treatment (Fig. S1). More importantly, the total numbers of Tr1 cells were increased (Fig. S1). Supporting the hypothesis that the IL-10 producers are generated de novo, since activation of CD4<sup>+</sup> T cells in the presence of IL-27 or TGF-β plus IL-27 results in the differentiation of IL-10-producing Tr1 cells (29, 30), we isolated total intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) from anti-CD3–treated and untreated mice to measure the mRNA expression level of these cytokines. Tgβ1, 2, 3 and Il27 mRNAs significantly increased in the proximal part of the small intestine (duodenum and jejunum) following anti-CD3 treatment (Fig. 2B), implying that Tr1 cells may be generated in response to these inducers in both locations.
Intestinal Tr1 cells have the ability to migrate and suppress diabetes development. To study the cellular target of Tr1 cells, we generated transgenic mice on the NOD background, in which IL-10 production is specifically blocked in T cells by the overexpression of the p55 subunit of the IL-10 receptor (IL-10R). Of note, these transgenic mice do not differ from NOD mice in their incidence of spontaneous diabetes. We injected 10 μg of anti-CD3 antibody for 5 consecutive days into recent-onset diabetic CD4–DN–IL-10R transgenic or nontransgenic NOD mice to compare the diabetes reversal rate for each group. In response to anti-CD3, diabetes remission was generally maintained within 6 wk after the onset of diabetes and treatment in NOD mice, although some of the treated mice experienced disease relapse when they were monitored for a longer period (Fig. 4). This phenomenon resembled what was seen in clinical trials, namely that anti-CD3 can mediate the extension of the honeymoon phase in newly diagnosed diabetic T1D patients (40).

To further confirm the migration ability of Tr1 cells, we rectally administered 0.5 × 10^6 sorted GFP^+ IL-10^+ CD4^+ T cells into NOD-scid mice and analyzed the egress of these cells 2 wk after injection. The majority of CD4^+ T cells were found in the spleen and lymph node (LN), with lesser amounts in the small intestine, colon, and pancreas tissue (Fig. 3E). Therefore, Tr1 cells not only can actively migrate from intestine into the circulation and further to lymphoid and nonlymphoid tissue, but also are able to cross an epithelial barrier from the luminal side.

**IL-10 Signaling in CD4^+ T Cells Is Critical in Controlling Diabetes Development.** To study the cellular target of Tr1 cells, we generated transgenic mice on the NOD background, in which IL-10 signaling is specifically blocked in T cells by the overexpression of a dominant-negative IL-10R under the CD4 promoter (CD4–DN–IL-10R). Of note, these transgenic mice do not differ from NOD mice in their incidence of spontaneous diabetes. We injected 10 μg of anti-CD3 antibody for 5 consecutive days into recent-onset diabetic CD4–DN–IL-10R transgenic or nontransgenic NOD mice to compare the diabetes reversal rate for each group. In response to anti-CD3, diabetes remission was generally maintained within 6 wk after the onset of diabetes and treatment in NOD mice, although some of the treated mice experienced disease relapse when they were monitored for a longer period (Fig. 4). This phenomenon resembled what was seen in clinical trials, namely that anti-CD3 can mediate the extension of the honeymoon phase in newly diagnosed diabetic T1D patients (40).

**IL-10 Signaling in CD4^+ T Cells Is Critical in Controlling Diabetes Development.** To study the cellular target of Tr1 cells, we generated transgenic mice on the NOD background, in which IL-10 signaling is specifically blocked in T cells by the overexpression of a dominant-negative IL-10R under the CD4 promoter (CD4–DN–IL-10R). Of note, these transgenic mice do not differ from NOD mice in their incidence of spontaneous diabetes. We injected 10 μg of anti-CD3 antibody for 5 consecutive days into recent-onset diabetic CD4–DN–IL-10R transgenic or nontransgenic NOD mice to compare the diabetes reversal rate for each group. In response to anti-CD3, diabetes remission was generally maintained within 6 wk after the onset of diabetes and treatment in NOD mice, although some of the treated mice experienced disease relapse when they were monitored for a longer period (Fig. 4). This phenomenon resembled what was seen in clinical trials, namely that anti-CD3 can mediate the extension of the honeymoon phase in newly diagnosed diabetic T1D patients (40).
diabetic patients (37, 38). Although the difference in the rate of diabetes reversion did not reach statistical significance in the 12-wk window between NOD mice and CD4–DN–IL-10R transgenic mice treated with anti-CD3 (47% in NOD vs. 25% in CD4–DN–IL-10R NOD, P = 0.1), the reversal rate is significantly higher in NOD mice within a time window of 6 wk (P = 0.03) (Fig. 4B).

Therefore, direct suppression of CD4 T cells via the IL-10-signaling pathway at least partially contributes to the effect of anti-CD3 treatment in vivo.

We found previously that Tr1 cells can control Th17 cells directly through the IL-10 receptor, which is expressed in the latter cells (19). To investigate if diabetogenic CD4 T cells also express IL-10R, we isolated CD4+CD25+ T cells from spleens of BDC2.5 NOD mice and found that these T cells expressed a high level of IL-10R. When mice became diabetic, about 30% of CD4 T cells expressed INF-γ, and these Th1 cells also expressed a significant amount of this cytokine receptor (Fig. 4C). Therefore, Tr1 cells in principle are able to control diabetogenic T cells directly in both a prediabetic environment and fully differentiated Th1 cells at the late stage of disease.

Tr1 Cells Generated in Vitro from Memory or Total CD4 T Cells Have a Different Capacity to Suppress Diabetes Development. To explore the therapeutic possibility of the use of Tr1 cells to treat autoimmune diabetes, we differentiated and expanded antigen-specific Tr1 cells in vitro and tested their stability and function in an adoptive transfer model. Total or memory CD4+ T cells were isolated from BDC2.5 TCR-transgenic NOD mice. Upon culture with IL-27 and TGF-β (30), 29.8 ± 4.55% IL-10–producing Tr1 cells were generated from memory CD4+ T cells, while only 8.84 ± 1.44% Tr1 cells were generated from total CD4+ T cells (Fig. 5A). This result is in line with previous findings (39) and suggests that memory T cells are the major source of this regulatory cell type. With regards to their suppressive function, Tr1 cells that were differentiated from total CD4+ T cells did not prevent diabetes (Fig. 5B), while Tr1 cells differentiated from memory CD4+ T cells significantly delayed disease development (Fig. 5C). Moreover, although BDC2.5 Teff cells isolated from CD4–DN–IL-10R transgenic NOD mice caused a similar diabetes incidence and secreted a comparable amount of IFN-γ as the normal BDC2.5 Teff cells (Fig. 5 C and D), Tr1 cells generated from the memory pool were no longer able to suppress these diabetogenic T cells, further confirming that Tr1 regulatory cells control the effector T cells via the IL-10–signaling pathway.

Due to the difference in suppressive function of Tr1 cells generated from two different sources of CD4 T cells, we further studied if cells differentiated from memory CD4 T cells are more stable in vivo. We sorted Tr1 cells differentiated from total or memory CD4+ T cells and then injected these cells into NOD-scid mice. Two weeks after injection, Tr1 cells were able to migrate into various tissues. Although the majority of the cells lost IL-10 expression, 5 to ~25% of IL-10 producer cells remained, independent of the original cellular source (Fig. S2A). This raised the issue of whether Tr1 cells might become pathogenic after loss of IL-10 expression. By intracellular staining, we observed that these exTr1 cells secrete IFN-γ and minimal amounts of IL-17 (Fig. S2B). Nevertheless, the loss of the ability to secrete IL-10 did not render those cells diabetogenic in NOD-scid mice in a 75-d observation window.

Discussion

In this paper, we highlight the importance of Tr1 cells and their suppressive mechanism in blocking diabetes development. Given that combinations of anti-CD3 mAb, autoantigen, and IL-10 treatment can revert autoimmune diabetes in NOD mice to a greater extent than monotherapy or any of the two-way combinations (11), it is possible that the synergy obtained through combined therapy may be due to increased induction of Tr1 cells. Although the mechanisms of anti-CD3 treatment for

**Fig. 4.** IL-10 signaling in CD4+ T cells is critical in controlling diabetes development. (A) Individual glycemia values of anti-CD3-treated recent-onset diabetic NOD mice (Left) and CD4–DN–IL-10R NOD mice (Right). Blood glucose concentrations were monitored until 12 wk posttreatment initiation. (B) Percentages of nondiabetic mice in NOD (n = 15) and CD4–DN–IL-10R NOD (n = 12) mice after anti-CD3 treatment. *P = 0.03. Statistical significance between groups was calculated using a log-rank (Mantel–Cox) test. (C) IL-10R expression was measured by flow cytometry. Cells were gated on CD4+CD25+ T cells, and Th1 (CD4+IFNγ+) cells were isolated from the spleen of a prediabetic and diabetic mouse. Results are representative of at least two independent experiments.

**Fig. 5.** In vitro-generated Tr1 cells have a different capacity in suppressing diabetes development. (A) Total CD4 and memory CD4 T cells were cultured for 5 d with anti-CD3 and anti-CD28 in the presence of mouse recombinant TGF-β and IL-27. Percentages of Tr1 cells were compared after in vitro differentiation. Statistical significance was determined by using a paired Student t test. Data are means ± SEM of four independent experiments. (B) BDC2.5 TCR-Tg CD4+CD25+ cells (1 × 104) were injected into NOD-scid mice either alone or co-injected with in vitro-differentiated Tr1 cells from total CD4 T cells. (C) BDC2.5 TCR-Tg CD4+CD25+ Teff cells (1 × 105) from wild-type or CD4–DN–IL-10R NOD mice were injected into NOD-scid either alone or co-injected with in vitro-differentiated Tr1 cells from memory CD4 T cells. Statistical significance was determined by log-rank (Mantel–Cox) test. (D) Cytokine production by CD4 T cells was analyzed intracellularly with flow cytometry and compared between wild-type and CD4–DN–IL-10R NOD mice. Percentages of the IFN-γ–producing T cells are indicated. Data are representative of three independent experiments.
autoimmune diabetes are also thought to involve Foxp3 T cells, the evidence has been conflicting (10, 40–42). In some studies, Foxp3 Tregs have been expanded to various degrees after a given treatment while, in others, Treg numbers were decreased. In our study, we also observed systematically reduced levels of Foxp3 T cells. Instead, the numbers of Tr1 cells were significantly increased, implying that this cell type may contribute significantly to the efficacy of the treatment.

It has been well recognized that, even with all these therapies, not all patients will respond. Data from clinical studies suggest that there are “responders” and “nonresponders” (43). Therefore, identification of the genetic, metabolic, and immunological features that differentiate responders and nonresponders may help to tailor therapies for subjects to improve efficacy and safety and to guide how combinations might be constructed. When monitoring children with newly diagnosed T1D for 3 mo, Sanda et al. (44) found that higher Foxp3 expression in T cells at diagnosis predicted worse future glycemic control, while higher mean numbers of IL-10+ T cells were associated with better future glucose control as measured by HbA1C. Therefore, quantifying IL-10+ T cell numbers might be a better way to distinguish the populations which might be at high risk for developing disease, identify trends, and serve as an immunological biomarker that could predict the treatment outcome.

Our study suggests that treatments preventing healthy or at risk people from developing disease is even more important. Because we showed that dysbiotic gut microbiota can induce intestinal Tr1 cells and that these cells also have the ability to migrate into the periphery and other organs, Tr1 cells may serve to patrol in the steady state to regulate pathogenic immune responses by suppressing, for example, autoreactive T cells that have escaped thymic deletion. Once immune tolerance is overcome, autimmune cells will react quickly and start to attack human tissues. Therefore, a deficit of Tr1 cells might be an underlying first trigger to initiate human autoimmune diseases. In fact, in newly diagnosed T1D patients and first-degree relatives, fewer antigen-specific IL-10–secreting cells were found compared with healthy controls (45, 46). Therefore, modulation of gut-associated lymphoid tissue (GALT) to boost Tr1 cells could represent a means to affect the natural history of autoimmune diabetes. Multiple strategies targeting mucosal tissue to modulate local and systemic immune responses have demonstrated success, such as oral administration of probiotic bacteria (47). Oral administration of a mixture of different strains of viable lyophilized probiotic bacteria in early diabetic NOD mice, including *Bifidobacteria*, *Lactobacillus*, and *Streptococcus salivarius* subsp. *sanguinis*, IL-10–producing cells in GALT increased islet destruction and the onset of clinical signs of diabetes. Interestingly, *Bifidobacterium* and *Lactobacillus* species are widely used in the food industry for production of yogurt and cheese, which are thought to be beneficial in reducing the risk of diabetes. By contrast, early exposure to a particular diet, such as cows’ milk (48, 49), gluten, and other cereal components (50, 51), may trigger or promote autoimmune reactivity. From this point of view, diet, which is thought to be beneficial in reducing the risk of diabetes.

Several studies have explored the potential of Tr1 cells as therapeutic agents in a number of settings (52–54). To explore the possibility of in vitro-expanded Tr1 cells as an adoptive cell therapy, we differentiated IL-10–producing cells from either total or memory CD4 T cells. Surprisingly, we found that only Tr1 cells generated from memory T cells could suppress diabetogenic T cells. However, no matter from which cell pool the regulatory cells were generated, both Tr1 populations showed lineage plasticity, similar to what has been previously reported for in vitro-expanded Foxp3 Tregs (55, 56). Cells that previously expressed IL-10, called eTr1 cells, acquired effector-like properties by producing cytokines like IFN-γ or IL-17. Although these cells did not elicit autoimmunity, at least in a 75-d observation window, further investigation of the stability, function, and phenotypic and genotypic characteristics of the different source of Tr1 cells will be essential to further secure the ongoing clinical trials.

### Materials and Methods

#### Mice.
NOD mice and BDC2.5 transgenic NOD mice were purchased from The Jackson Laboratories. Foxp3–/– (S7) IL-10–/– double-reporter mice (20) and dominant-negative IL-10R mice (CD4–DN–IL-10R) (58) were backcrossed to a NOD background for 10 generations. Age- and sex-matched littermates between 8 and 16 wk old were used.

Inflammase-deficient mice (Asc–/– and Nlrp3–/–) with dysbiotic gut microbiota were used for the cohosting experiments. Female diabetes-prone NOD mice or Foxp3+IL-10−/− double-reporter NOD mice were cohosted with dysbiotic mice at a 1:1 ratio for 3–6 mo. All animal procedures were approved by the Institutional Animal Care and Use Committee of Yale University.

#### Intestinal Lymphocyte Isolation.
Mice were injected with anti-CD3 (10–15 μg per mouse, 2C11) or PBS intraperitoneally two times every other day. After removal of the Peyers’s Patches, IEL and LPL were isolated via incubation with trypsin, EDTA, and collagenase from Clostridium histolyticum (2139; Sigma) and DNase at 37 °C for 1 h (for LPL). Cells were then further separated with a Percoll gradient.

#### Flow Cytometry.
Cell suspensions were prepared from spleen, lymph nodes, pancreas, and intestine of control mice or mice treated with anti-CD3. Samples were stained with fluorochrome-labeled mAbs against cell-surface antigens and analyzed on a LSRII flow cytometer (BD Biosciences). The following mAbs were used: anti-CD4 (RM4-4), TCR-μ (H57-597), IL-10R (10B.1.3a), IL-10R (H57-597), IL-4 (12G1), CCR6 (HM-CRS), CCR7 (AB12), CCR9 (OV-12), and IgG isotype Ctrl (HTK888), Biotin Rat IgG2a, κ isotype Ctrl (RTK2758), mouse IgG2a, and κ isotype Ctrl (MOPC-173). All mAbs were from BioLegend, and data were analyzed using FlowJo.

For intracellular cytokine staining, the cells were restimulated for 4 h at 37 °C with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml; Sigma) and ionomycin (1 μg/ml; Sigma) in the presence of GolgiStop (BD Biosciences). Cells were then fixed and permeabilized with BD Cytofix/Cytoperm buffer and stained at 4 °C with anti-IL-17A (catalog no. S60184; BD Bioscience) and anti-IFNγ (catalog no. 554412; BD Bioscience) antibodies for 30 min. Lymphocytes were resuspended in PBS and 0.5% FBS.

#### Adoptive Transfer.
CD4+CD25+ Teff cells and CD4+Foxp3 IL–10+ (Tr1) cells were FACs-sorted from spleen and intestine of BDC2.5 double-reporter NOD mice, respectively. A total of 100,000 Teff cells were i.v. injected into to 6-wk-old NOD-scid recipients with or without the same number or five times fewer of Tr1 cells. Disease development was monitored by testing the urine glucose.

#### In Vitro Tr1 Cell Differentiation.
We FACs-sorted total or memory CD4+ T cells (CD44hiCD62Llo) and activated them with plate-bound monoclonal anti-CD3 (1 μg/ml; BD Biosciences, #377) at 37 °C for 3 d in 10% FBS (for IEL), followed by further digestion with collagenase from *Clostridium histolyticum* (HTK888), Biotin Rat IgG2a, κ isotype Ctrl (RTK2758), mouse IgG2a, and κ isotype Ctrl (MOPC-173). All mAbs were from BioLegend, and data were analyzed using FlowJo.

#### RT-PCR.
Inflammation was measured by RT-PCR for the 15 genes described above.

#### RT-PCR.
CD4+CD25+ Teff cells and CD4+Foxp3 IL–10+ (Tr1) cells were FACs-sorted from spleen and intestine of BDC2.5 double-reporter NOD mice, respectively. A total of 100,000 Teff cells were i.v. injected into to 6-wk-old NOD-scid recipients with or without the same number or five times fewer of Tr1 cells. Disease development was monitored by testing the urine glucose.

#### In Vitro Tr1 Cell Differentiation.
We FACs-sorted total or memory CD4+ T cells (CD44hiCD62Llo) and activated them with plate-bound monoclonal anti-CD3 (1 μg/ml; BD Biosciences, #377) at 37 °C for 3 d in 10% FBS (for IEL), followed by further digestion with collagenase from *Clostridium histolyticum* (HTK888), Biotin Rat IgG2a, κ isotype Ctrl (RTK2758), mouse IgG2a, and κ isotype Ctrl (MOPC-173). All mAbs were from BioLegend, and data were analyzed using FlowJo.

#### Adoptive Transfer.
CD4+CD25+ Teff cells and CD4+Foxp3 IL–10+ (Tr1) cells were FACs-sorted from spleen and intestine of BDC2.5 double-reporter NOD mice, respectively. A total of 100,000 Teff cells were i.v. injected into to 6-wk-old NOD-scid recipients with or without the same number or five times fewer of Tr1 cells. Disease development was monitored by testing the urine glucose.

#### Intrarectal Administration of Tr1 Cells.
IL-10 eGFP+ Tr1 cells were sorted after in vitro differentiation. NOD-scid recipient mice were fasted overnight before cell administration. The next day, mice were anesthetized with an intraperitoneal injection of ketamine/xylazine and then intrarectally injected with 0.5 × 106 cells with a gavage needle. Mice were monitored until full recovery and maintained in fasting for another night. Two weeks later, mice were euthanized to assess the T cell distribution.

#### mAb Treatment and Blood Glucose Monitoring.
Anti-mouse CD3 mAb 10 μg (145–2C11) was administered i.p. in newly onset diabetic mice for 5 consecutive days. A diagnosis of diabetes was made when mice had blood glucose levels of at least 250 mg/dl on two consecutive occasions. Blood glucose
was measured in the morning twice a week using a Turebalance Glucose Meter (NIPRO Diagnostics). Diabetes remission was defined as the absence of glycosuria, and glycaemia values >250 mg/dl never occurred in any of the treated mice.

Statistical Analysis. Differences in diabetes incidence were assessed using the Mantel–Cox log-rank test. Statistical significance of other comparisons was tested using paired or unpaired two-tailed Student’s t test or two-way ANOVA (multiple comparisons test) as indicated. Graphs were plotted and statistics calculated with GraphPad Prism v. 4.00 for Macintosh (GraphPad Software).

ACKNOWLEDGMENTS. We thank C. Lieber, E. Hughes-Picard, and J. Alderman for expert administrative assistance and B. Hu and H. Xu for technical help and scientific discussion. This work was supported by NIH Grant R01 DK 51665 (to R.A.F.); Yale Diabetes Research Center Grant P30 DK045735; the American Diabetes Association Research Foundation Postdoctoral Fellowship (to H.Y.); and the Dr. Keith Landesman Memorial Fellowship of the Cancer Research Institute (to N.G.). R.A.F. is an investigator of the Howard Hughes Medical Institute.