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Regulatory T Cells Prevent Transfer of Type 1 Diabetes in NOD Mice Only When Their Antigen Is Present In Vivo

Daniel R. Tonkin, Jing He, Gene Barbour, and Kathryn Haskins

Regulatory T cells (Tregs) can potentially be used as tools to suppress pathogenic T cells in autoimmune diseases such as type 1 diabetes. For use in therapy it is critically important to determine whether suppression by Tregs requires a population specific for the target of autoimmunity, such as pancreatic β cells in type 1 diabetes. Current reports in the NOD mouse model of type 1 diabetes are in conflict as to whether suppression of disease by Tregs is Ag-dependent. We have addressed this question by evaluating the effects of islet-specific TGF-β-induced Tregs in recipient mice in which the Treg Ag is either present or absent. Our data show that Treg numbers in pancreas are reduced in the absence of Ag and that there are Ag-dependent differences in the effects of Tregs on pathogenic T cells in the pancreas. By examining protection from diabetes induced by T cell transfer, we have clearly demonstrated that Tregs suppress only in the presence of their Ag and not in mice in which the islets lack the Treg Ag. Our results also suggest that in sufficiently large populations of polyclonal Tregs, there will be adequate numbers of islet-specific Tregs to suppress diabetes. The Journal of Immunology, 2008, 181: 4516–4522.
in islets from other mouse strains (21). Based on the restriction of the BDC-6.9 TCR reactivity to NOD islets, a gene controlling the Ag was mapped to a locus on chromosome 6 (22). A congenic mouse, the NOD.C6, was bred such that this locus was replaced with BALB/c DNA (21), and as a result T cells expressing the BDC-6.9 TCR cannot respond to NOD.C6 islets and cannot induce diabetes in the NOD.C6 mouse, which otherwise develops diabetes at a similar rate as NOD mice. By using the NOD.C6 mouse, we could test the ability of 6.9 TCR-Tg/NOD Tregs to protect against adoptive transfer of diabetes in the absence of their islet Ag. This study is the first to evaluate tissue-specific suppression by the same TCR-Tg Tregs in the presence and absence of Ag in vivo, and our results provide a clear demonstration of the in vivo Ag dependence of Tregs. We also report on Ag-dependent differences in Treg trafficking in vivo and on the suppression of Th1 effector cell cytokines in an Ag-deficient pancreas.

**Materials and Methods**

**Mice**

NOD and NOD.scid breeding mice were initially acquired from The Jackson Laboratory or the Barbara Davis Center for Childhood Diabetes (Denver, CO) and were housed in specific pathogen-free conditions at the University of Colorado Denver (UCD) Center for Laboratory Animal Care (CLAC). Experimental animals were monitored for development of disease by urine glucose (Diastix, Bayer) and hyperglycemia confirmed by One-Touch Ultra glucometer (LifeScan). Mice were considered diabetic when blood glucose levels were >15 mmol/l (270 mg/dl). The 2.5 TCR-Tg/NOD and 6.9 TCR-Tg/NOD mice were produced using TCR genes from diabetic T cell clones BDC-2.5 and BDC-6.9, respectively (21, 23). NOD congenic mice lacking the BDC-6.9 Ag (NOD.C6) were produced as previously described (21) and were crossed with 6.9 TCR-Tg/NOD mice to produce 6.9 TCR-Tg/NOD.C6 mice.

The 2.5 TCR-Tg/NOD mouse (hereafter referred to as 2.5 TCR-Tg) was used as a source of Th1 effector T cells. The T cells from the 2.5 TCR-Tg are different in their Ag specificity and can be distinguished from 6.9 TCR-Tg T cells by means of a clonotypic Ab (Ab 24) to the BDC-2.5 TCR. Because 6.9 TCR-Tg/NOD.C6 (hereafter referred to as 6.9 TCR-Tg) mice do not become diabetic and are therefore easier to maintain, the 6.9 TCR-Tg on the NOD.C6 background was routinely used as a source of CD4 T cells for Tregs instead of 6.9 TCR-Tg/NOD mice; moreover, Tregs derived from both strains were indistinguishable from one another. All procedures were in accordance with Institutional Animal Care and Use Committee guidelines and approved by the UCHSC Animal Care and Use Committee.

**Induction of Th1 cells and Tregs**

Th1 cells and induced Tregs were generated by a protocol similar to one previously described (13). CD4 T cells were purified from 2- to 3-mo-old NOD, 2.5 TCR-Tg, or 6.9 TCR-Tg mice by harvesting spleen and lymph nodes, followed by positive selection with magnetic anti-CD4 microbeads (Miltenyi Biotec). CD4 T cells were reconstituted at 1 × 10^6 cells/ml in complete medium, which is DMEM supplemented with 44 mM sodium bicarbonate, 0.55 mM t-serine, 0.27 mM t-asparagine, 1.5 mM t-glutamate, 1 mM sodium pyruvate, 50 mM 2-ME, 10 mM HEPES, and 10% FCS. T cells were activated by placing 5 × 10^6 cells per well in 6-well tissue culture plates coated with 1 μg/ml anti-CD3 (BD Biosciences). For Th1 cells, 100 U/ml of recombinant human IL-2 (National Cancer Institute) was added to the media. For induction of Tregs, medium was supplemented with 100 U/ml IL-2, 3 ng/ml human TGF-β1 (PeproTech), and 2.5% anti-IFN-γ antibody from the XMG1.2 (BioLegend), a level similar to that used for mice in each experimental group. After digestion and homogenization, cells from each group were divided in half and cultured in the presence of GolgiPlug for 4–5 h at 37°C with no stimulation or in wells containing anti-CD3 (5 μg/ml) and anti-CD28 (5 μg/ml). Cells were then harvested and stained for surface markers and intracellular cytokines.

**Statistical analysis**

Statistical significance of the effect of T cell treatments (Tregs, CD4 T cells) on diabetes transfer was determined by a Wilcoxon test of survival among mice in each experimental group. After digestion and homogenization, cells from each group were divided in half and cultured in the presence of GolgiPlug for 4–5 h at 37°C with no stimulation or in wells containing anti-CD3 (5 μg/ml) and anti-CD28 (5 μg/ml). Cells were then harvested and stained for surface markers and intracellular cytokines.

**Results**

Tregs induced from TCR-Tg NOD mice with TGF-β express Foxp3 and produce little IFN-γ

It has been previously reported that T cells from the 2.5 TCR-Tg mouse can be induced to express Foxp3, either through retroviral transduction (19) or in vitro culture with TGF-β (13). To confirm that the Tregs generated from CD4 T cells of the 6.9 TCR-Tg mouse expressed Foxp3, we incubated CD4 T cells isolated from these mice either under Th1- or Treg-promoting conditions, as described in Materials and Methods. After 3 days, cells were harvested and Foxp3 protein production was evaluated by intracellular staining. Our results indicated that <10% of T cells in the “Th1” population expressed Foxp3 (Fig. 1A), a level similar to what is observed with natural Tregs in unmanipulated populations.
ment for Treg Ag during this process, we tested the IFN-
-Th1 T cells in vitro and to determine whether there was a require-

addition of anti-IFN-

levels of IL-10 and TGF-

of cytokines. Although this difference between the two TCR-Tg

cells from 6.9 TCR-Tg/NOD mice generally produce lower levels

duced from 2.5 TCR-Tg/NOD mice (13), we have found that T

ers. The 2.5 TCR-Tg Th1 T cells were placed in culture with islet cells

T cells are undefined, the two clones respond to different epitopes.

To demonstrate the ability of TGF-

production was analyzed by flow cytometry. B, After harvest

and a 3-day expansion with IL-2, 6.9 TCR-Tg Th1 cells and Tregs were

activated with anti-CD3 (1

and TGF-

Foxp3 is up-regulated and IFN-

Th1 cells and Tregs were activated for 3 days

with anti-CD3 (1 μg/ml) and IL-2 for Th1 cell production, and with the

addition of anti-IFN-γ and TGF-β for induction of Tregs. Intracellular

Foxp3 protein production was analyzed by flow cytometry. B, After harvest

and a 3-day expansion with IL-2, 6.9 TCR-Tg Th1 cells and Tregs were

activated with anti-CD3 (1 μg/ml) and cytokine production was measured

by intracellular staining.

In cells that underwent Treg induction, however, Foxp3 was ex-

pressed in 80–95% of the T cells (Fig. 1A). Th1 cells and Tregs

were activated with anti-CD3, and intracellular staining showed that IFN-γ production was greatly reduced in the Treg population

(Fig. 1B). This robust induction of Foxp3, accompanied by reduc-

ion of IFN-γ, demonstrates that in vitro treatment of 6.9 TCR-Tg

T cells with TGF-β leads to the induction of T cells with a Treg

phenotype.

We also tested for production of two suppressive cytokines,

TGF-β and IL-10. Although TGF-β production was increased in

our Treg population (Fig. 1B), this effect was variable and in some

assays was difficult to detect. We did observe low levels of IL-10 in

the TGF-β-induced Tregs, but these levels were similar to those

observed in the Th1 population (data not shown). While others

have observed greater TGF-β and IL-10 production by Tregs indi-

duced from 2.5 TCR-Tg/NOD mice (13), we have found that T

cells from 6.9 TCR-Tg/NOD mice generally produce lower levels

cytokines. Although this difference between the two TCR-Tg

mouse strains is not fully understood, it may account for the lower

levels of IL-10 and TGF-β production by 6.9 TCR-Tg Tregs.

**FIGURE 1.** Foxp3 is up-regulated and IFN-γ is decreased in induced Tregs. A, CD4 T cells from a 6.9 TCR-Tg mouse were activated for 3 days

with anti-CD3 (1 μg/ml) and IL-2 for Th1 cell production, and with the

addition of anti-IFN-γ and TGF-β for induction of Tregs. Intracellular

Foxp3 protein production was analyzed by flow cytometry. B, After harvest

and a 3-day expansion with IL-2, 6.9 TCR-Tg Th1 cells and Tregs were

activated with anti-CD3 (1 μg/ml) and cytokine production was measured

by intracellular staining.

**FIGURE 2.** TGF-β-induced Tregs suppress IFN-γ production by Th1 T

cells in vitro only in the presence of Treg Ag. The 2.5 TCR-Tg Th1 cells

or 6.9 TCR-Tg Tregs (1 × 10⁴) were activated with NOD or NOD.C6 islets

(1 × 10⁴) as a source of Ag, and thioglycollate-elicited peritoneal exudate

cells (5 × 10⁴) as APC, in 96-well plates. For co-cultures of Th1 cells and

Tregs, 1 × 10⁵ cells of each T cell type were cultured together. After 24 h,

IFN-γ in the supernatant was measured by ELISA. Error bars indicate SD

from the mean of triplicate cultures, and data are representative of three

experiments.

***TGF-β-induced Tregs suppress diabetes transfer in both immunodeficient and immunocompetent recipients***

To demonstrate that TGF-β-induced Tregs obtained from the

BDC-6.9 TCR-Tg mouse could effectively protect against adoptive

transfer of diabetes in either immunodeficient NOD.scid recipients

or in immunocompetent NOD mice, we performed experiments in

which 6.9 TCR-Tg Tregs were cotransferred with 2.5 TCR-Tg Th1

cells. As illustrated in Fig. 3A, the 2.5 TCR-Tg Th1 T cells rapidly

transferred diabetes in NOD.scid recipients. In these mice, after
cotransfer of 6.9 TCR-Tg Tregs with 2.5 TCR-Tg Th1 cells at a 5:1 ratio,
diabetes was completely suppressed for up to 6 mo (p = 0.0008).

Protection was also achieved for at least 3 mo when Tregs were

used at a 2:1 ratio (data not shown). There was no protection,

however, when 6.9 TCR-Tg CD4 T cells without TGF-β induction were

used in cotransfers with the Th1 cells, indicating that coinjection of untreated transgenic T cells was not adequate to suppress
disease.

Similar experiments were conducted in immunocompetent NOD

mice, a more physiologically relevant model. In these recipients

transfer was somewhat less consistent and greater numbers of 2.5

TCR-Tg Th1 cells (1 × 10⁵) were required to achieve rapid in-
duction of diabetes in most recipients (Fig. 3B). In cotransfers with

6.9 TCR-Tg Tregs (3 × 10⁴), diabetes was prevented in most of

the young NOD mice (p = 0.004), demonstrating the ability of

TGF-β-induced Tregs to effectively suppress diabetes in an

immunocompetent animal.
Tregs suppress cytokine production by pancreas-infiltrating effector T cells

In previous studies, we have demonstrated that upon adoptive transfer of diabetogenic Th1 T cell clones to NOD.scid mice, substantial amounts of inflammatory cytokines such as IFN-γ and TNF can be detected ex vivo in T cells isolated from the pancreas (25). To explore the mechanism by which Tregs protect against pathogenic T cells infiltrating the pancreas, we performed ex vivo analysis of Th1 T cells retrieved from the pancreas of NOD.scid recipients following adoptive transfers of 2.5 TCR-Tg Th1 T cells either alone or in the presence of 6.9 TCR-Tg Tregs. Six to 9 days following transfers, pancreata were harvested, digested with collagenase, and mechanically homogenized to isolate infiltrating leukocytes. Both Th1 cells and Tregs were observed within the pancreas, and we evaluated cytokine production of pancreas-infiltrating T cells by stimulating the cells immediately after harvest with plate-bound anti-CD3 Ab in the presence of GolgiPlug (brefeldin A) to prevent cytokine secretion. Intracellular cytokines were detected with fluorescent Abs by flow cytometry and the Th1 T cell population was identified using a clonotypic Ab to the BDC-2.5 TCR. As illustrated in Fig. 4, we observed that the percentages of Th1 cells producing IFN-γ and TNF were reduced at all time points in mice that received cotransfers of Th1 T cells and Tregs. These results indicate that one effect of Tregs in the pancreas is a reduction in the percentage of Th1 cells producing inflammatory cytokines. In data not shown here, there was no decrease in numbers of pancreas-infiltrating Th1 cells until later time points, suggesting that the reduction in cytokine production is not due to Tregs preventing Th1 survival or migration to the pancreas. Rather, it appears that the Tregs suppressed the cytokine production of Th1 cells recruited to the pancreas, but it is not yet clear if this effect requires Treg activity in the pancreas, the draining lymph node, or both.

Protection by polyclonal Tregs is effective only with large numbers of Tregs

Because human Tregs must be derived or isolated from a polyclonal source, the efficacy of polyclonal Tregs is of particular therapeutic relevance, but reports of the suppressive capacity of polyclonal Tregs in NOD mice have been variable (9, 11–12, 18 –20). We assessed whether Tregs with diverse TCR Ag specificities could suppress adoptive transfer of diabetes by carrying out cotransfers of TCR-Tg Th1 T cells with TGF-β-induced Tregs obtained from polyclonal populations of NOD CD4 T cells. We injected NOD.scid mice with 2:1 and 5:1 ratios of polyclonal NOD Tregs to 2.5 TCR-Tg Th1 cells. Fig. 5 shows that at a 2:1 ratio, the polyclonal Tregs appeared completely unable to suppress, whereas at a 5:1 ratio polyclonal Tregs provided protection in about half of the recipients. Our results are consistent with the conclusion that polyclonal Tregs are protective only if sufficient numbers of Ag-specific Tregs are contained within the Treg population, and they may help to explain the variable results on efficacy of polyclonal Tregs reported in the literature.

TGF-β-induced Tregs fail to protect in vivo in the absence of their Ag

To evaluate the in vivo Ag dependence of TGF-β-induced Tregs during suppression of diabetes, we compared protection of Tregs obtained from 2.5 TCR-Tg or 6.9 TCR-Tg mice in NOD.C6 recipients in which the islet Ag for the BDC-2.5 TCR is present but is lacking for the BDC-6.9 TCR. As presented in Fig. 6, 2.5 TCR-Tg Th1 cells induced diabetes in most of the recipients, and in cotransfers with 2.5 TCR-Tg Tregs, transfer of disease was
completely suppressed \((p = 0.007)\). In contrast, in the cotransfers of 2.5 TCR-Tg Th1 effectors and 6.9 TCR-Tg Tregs, there was no significant delay of diabetes \((p = 0.12)\). As 6.9 TCR-Tg Tregs prevent diabetes induction in NOD mice but not in Ag-negative NOD.C6 mice, these results provide clear evidence that TGF-β-induced Tregs require in vivo antigenic stimulation to suppress diabetes.

Numbers of TGF-β-induced Tregs in the pancreas are greater in the presence of Treg Ag

It is not known how the in vivo behavior of Tregs is altered in the absence of Ag. To address this question, we labeled 6.9 TCR-Tg Tregs with CFSE before transfer into NOD and NOD.C6 recipients to compare Treg migration in the presence and absence of Ag. Four days after transfer, the pancreas, pancreatic lymph nodes, and distal lymph nodes were harvested from each mouse strain. Fig. 7 indicates that similar numbers of 6.9 TCR-Tg Tregs were present in distal lymph nodes and pancreatic lymph nodes of both NOD and NOD.C6 mice. Transferred cells in these lymphoid organs had undergone minimal proliferation. Similar results were observed in the spleen (data not shown). In contrast, in the pancreata of NOD.C6 mice the numbers of CFSE-positive 6.9 TCR-Tg Tregs were considerably smaller than those found in NOD pancreata.

Despite the lack of Ag in the NOD.C6 mice, the 6.9 TCR-Tg Tregs did undergo several rounds of proliferation in the pancreas of both NOD and NOD.C6 mice, possibly due to the presence of IL-2 produced by preexisting inflammation. It is clear, however, that in the pancreas the numbers of 6.9 TCR-Tg Tregs are markedly affected by the presence of Ag, whether due to reduced migration of Tregs or to decreased retention of Tregs in the Ag-deficient pancreas.

Suppression of effector Th1 T cells by TGF-β-induced Tregs occurs only in the presence of Treg Ag

In our results shown in Fig. 4, we observed that the percentages of Th1 cells producing IFN-γ and TNF were consistently reduced in NOD.\(\text{scid}\) mice that received cotransfers of Th1 T cells and Tregs, indicating that one mechanism by which Tregs protect is through suppression of cytokine production by pathogenic Th1 T cells in the pancreas. To identify Ag-dependent effects of in vivo suppression, we compared the suppression of cytokine production in 2.5 TCR-Tg Th1 cells by 6.9 TCR-Tg Tregs in the pancreas of Ag-positive NOD and Ag-negative NOD.C6 mice. The 2.5 TCR-Tg Th1 cells were injected into NOD and NOD.C6 mice alone or in the presence of 6.9 TCR-Tg Tregs. We harvested the pancreata on day 5 or 6 and measured cytokine production after in vitro stimulation with anti-CD3 and anti-CD28 Abs. As illustrated in Fig. 8, 6.9 TCR-Tg Tregs caused a modest decrease in TNF production by Th1 effectors in the NOD recipients. In the NOD.C6 recipients, however, there was little to no suppression of TNF, indicating that...
Tregs require their Ag to inhibit TNF production in the pancreas. A similar effect was observed for IFN-γ, but these results were somewhat more variable (data not shown). Taken together, these results provide strong evidence that Tregs suppress cytokines by Th1 effectors only if the Treg Ag is present.

Discussion
We have reported herein on the protective activity of Tregs generated through in vitro culture of CD4 T cells from the BDC-6.9 TCR-Tg mouse, the second transgenic mouse produced from a diabetogenic TCR of a CD4 T cell clone from our BDC panel (26). TGF-β-induced Tregs from the BDC-2.5 TCR-Tg mouse were shown by Weber et al. to protect both against adoptive transfer by BDC-2.5 TCR-Tg Th1 effector T cells and development of spontaneous disease in NOD mice (13). In this study, we exploited the restricted islet Ag specificity of the BDC-6.9 T cells to investigate the Treg requirement for the presence of Ag in vivo. Our results demonstrate conclusively that suppression of pathogenic Th1 T cell responses by TGF-β-induced Tregs occurs in an Ag-dependent manner. While our studies have taken advantage of the largely clonal populations of T cells provided by TCR-Tg mice, our results indicate that through induction of Tregs with TGF-β, polyclonal populations of T cells from nontransgenic NOD mice can also protect when used in sufficiently high numbers. Of greater note, our data provide the first demonstration of how Tregs function in vivo in the presence or absence of the Treg Ag.

For suppression of diabetes by Tregs specific for islet cell Ag, the requirement for Ag was clearly shown in NOD.C6 mice that lack the Ag for the BDC-6.9 T cell clone. TGF-β-induced Tregs from the 6.9 TCR-Tg mouse could not suppress diabetes in these animals, were reduced in number in the pancreas after adoptive transfer, and did not alter cytokine production by transferred Th1 T cells. Although the mechanism by which TGF-β-induced Tregs function is not well understood, it is clear that the suppressive effects of Tregs are Ag-dependent. Additionally, our experiments with TGF-β-induced Tregs obtained from polyclonal NOD CD4 T cells provide some explanation for the inconsistent results obtained with polyclonal Tregs in other reports. We showed that polyclonal Tregs transferred into NOD.scid mice could protect at sufficiently high Treg/Th1 ratios (5:1), but were not effective at lower numbers (e.g., 2:1), suggesting that the proportion of Ag-specific Tregs present in a polyclonal population dictates effective suppression of Th1 activity.

Our data also provide some explanation of how Tregs may operate in vivo, depending on whether their Ag is present in the inflammatory site. For example, our results demonstrate that accumulation of TGF-β-induced Tregs in the pancreas is largely dependent on the presence of Treg Ag in the pancreas. Although 6.9 TCR-Tg Tregs could be found in the Ag-negative NOD.C6 pancreas, their numbers were only about one-fourth of what was observed in an Ag-positive NOD pancreas, due either to decreased migration to the pancreas or to decreased retention of cells within the pancreas. The Tregs were transferred into recipients that were ~6 wk old, and pancreatic infiltration by host cells could already be observed in uninjected mice of this age. We hypothesize that Tregs migrated to the pancreas in response to chemokines produced by the infiltrating host cells. This scenario could also help to explain why Tregs were dividing only in the pancreas, and not in other tissues of both NOD and NOD.C6 recipients; we speculate that IL-2 produced by host T cells in the pancreas could be driving Ag-independent proliferation of Tregs at this site. Our results furthermore demonstrate that merely the presence of TGF-β-induced Tregs in an inflammatory site is not enough to trigger suppressive activity. Only through activation of the TCR in response to Ag can these Tregs suppress inflammation.

The in vivo requirement for Ag by TGF-β-induced Tregs indicates that use of Tregs for human therapy will likely require use of Ag-specific Tregs. Transfer of polyclonal Tregs would be the simplest approach, but a polyclonal population is likely to have limited effect on a specific organ. Additionally, it is possible that transfer of large numbers of polyclonal Tregs into a patient might result in undesired suppression of immunity toward pathogens and tumor Ags. It might be possible to isolate Ag-specific Tregs in humans by...
a protocol similar to that demonstrated in wild-type NOD mice in which a tetramer specific for the BDC-2.5 TCR was used to isolate and expand islet-specific Tregs (27). However, production of peptide-MHC complexes that can be used to isolate and stimulate T cells specific for target Ags in every patient is unlikely to be feasible. A more practical approach may be to induce islet Ag-specific Tregs from a polyclonal population through in vitro culture with TGF-β. Although activation of TGF-β-induced Tregs with anti-CD3 Abs has been the most common approach, Tregs have also been generated from TCR-Tg mice in response to peptide Ag (8, 28). Another report has shown that allogeneic dendritic cells can specifically expand alloreactive natural Tregs out of a polyclonal T cell population (29), demonstrating enrichment of a Treg subset based on Ag specificity, albeit a relatively large subset. Ideally, it might be possible to obtain Tregs specific to a single Ag out of a polyclonal population by activating T cells in the presence of TGF-β with Ag-loaded dendritic cells. Our results demonstrating in vivo suppression of 2.5 TCR-Tg Th1 T cells by 6.9 TCR-Tg Tregs, which recognize a different Ag epitope, are consistent with in vitro data demonstrating bystander suppression by Tregs (15, 16). This could be advantageous in therapy, because Tregs with specificity for one organ-specific Ag should be adequate to suppress T effectors with a wide range of Ag specificities within that organ.

Disclosures

The authors have no financial conflicts of interest.

References