Bystander Central Memory but Not Effector Memory CD8 $^{+}$ T Cells Suppress Allograft Rejection

Ni Wan, Hehua Dai, Tao Wang, Yolonda Moore, Xin Xiao Zheng and Zhenhua Dai

J Immunol 2008; 180:113-121; ;
doi: 10.4049/jimmunol.180.1.113
http://www.jimmunol.org/content/180/1/113

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Bystander Central Memory but Not Effector Memory CD8$^+$ T Cells Suppress Allograft Rejection[1]

Ni Wan,* Hehua Dai,* Tao Wang,* Yolonda Moore,* Xin Xiao Zheng,† and Zhenhua Dai[2]*

Memory T cells respond faster and more vigorously than their naive counterparts and are critical for adaptive immunity. However, it is unknown whether and how memory T cells react in the face of irrelevant Ags. It is generally accepted that bystander memory T cells are neutral in immune responsiveness. In this study, we present the first evidence that bystander central memory (T_{CM}), but not effector memory (T_{EM}), CD8$^+$ T cells suppress allograft rejection as well as T cell proliferation in the draining lymph nodes (DLN) of recipient mice. Both bystander T_{CM} and naive T cells, but fewer T_{EM} cells, migrated to DLN, whereas T_{CM} cells exhibited faster turnover than their naive counterparts, suggesting that bystander T_{CM} cells have an advantage over their naive counterparts in suppression. However, bystander T_{EM} cells migrated to inflammatory graft sites, but not DLN, and yet failed to exert their suppression. These findings indicate that bystander memory T cells need to migrate to lymph nodes to exert their suppression by inhibiting responder T cell activation or homeostatic proliferation. Moreover, the suppression mediated by bystander T_{CM} cells was largely dependent on IL-15, as IL-15 was required for their homeostatic proliferation and T_{CM}-mediated suppression of allograft rejection. This suppression also required the presence of TGFβ1, as T_{CM} cells expressed TGFβ1 while neutralizing TGFβ1 abolished their suppression. Thus, bystander T_{CM}, but not T_{EM}, CD8$^+$ T cells are potent suppressors rather than bystanders. This new finding will have an impact on cellular immunology and may have clinic implications for tolerance induction. ***The Journal of Immunology,*** **2008, 180: 113–121.**

A cardinal feature of an adaptive immune response is its ability to generate long-lasting populations of memory T cells (1–3). Memory T cells are specific to the Ag encountered during a primary immune response and respond rapidly and vigorously upon re-encounter with the same Ag. They are resistant to conventional costimulation blockade (4–7), though susceptible to 4-1BB and OX40 costimulation blockade (8, 9), function independently of secondary lymphoid organs (10, 11), and hinder allograft survival or tolerance induction (11–20). However, the role of bystander memory T cells in immune responsiveness is unclear. It is generally believed that they are neutral just as a bystander in an Ag-specific immune response. Another feature of adaptive immunity is the generation of regulatory T (Treg)[3] cells (21, 22). Treg cells prevent autoimmune diseases, suppress allograft rejection or graft-vs-host disease (23, 24), and are essential for the maintenance of tolerance (25–32). Like professional FoxP3$^+$ Treg cells (33, 34), memory T cells suppress lymphopenia-induced homeostatic proliferation of naive T cells (35). Therefore, bystander memory T cells could also act as Treg cells to suppress an Ag-specific immune response. Adversely, bystander memory T cells may help enhance an immune response, because an inflammatory site contains rich growth factors and cytokines that could activate bystander memory T cells, which in turn help promote the immune-based inflammation. In this study, we investigated the role of bystander memory CD8$^+$ T cells in an alloimmune response and demonstrated that they are not true bystanders. We found that bystander central memory (T_{CM}), but not effector memory (T_{EM}), CD8$^+$ T cells suppress allograft rejection as well as T cell proliferation in vivo. This suppression was largely dependent on IL-15 and TGFβ1, as IL-15 was required for the homeostasis of T_{CM} cells while neutralizing TGFβ1 abolished their suppression of allograft rejection.

Materials and Methods

Mice

The 2C TCR-transgenic C57BL/6 mice on a recombination activating gene-1 knockout (Rag1$^{-/-}$) background (2C.Rag$^{-/-}$) were generated by backcrossing 2C transgenic mice onto Rag1$^{-/-}$ mice (The Jackson Laboratory) (6, 14). Wild-type (WT) BALB/c, C3H/HeJ, and C57BL/6 mice were purchased from the National Cancer Institute (National Institutes of Health, Bethesda, MD). All mice were housed in a specific pathogen-free environment, and all animal protocols and experiments were approved by the Animal Care and Use Committee of the University of Texas Health Center.

Pancreatic islet transplantation

Islet donors were 7–8-wk-old C3H/HeJ female mice. Islet recipients were 7–8-wk-old Rag1$^{-/-}$ (B6 background) female mice. Islets were isolated and transplanted into the subcapsular space of the right kidney of recipient mice as described previously (14). Recipient mice were rendered diabetic by a single injection of streptozotocin (Sigma-Aldrich) (180 mg/kg) 10–14 days before transplantation. Primary graft function was defined as blood glucose under 200 mg/dl for 48 h after transplantation. Graft rejection was defined as a rise in blood glucose to >300 mg/dl for three consecutive days after primary function.

Memory T cell preparation and phenotyping

To generate memory CD8$^+$ T cells, naive CD8$^+$ T cells (CD8$^+$CD25$^-$ CD44^{low}) from transgenic 2C Rag$^{-/-}$ mice were isolated by a FACSAria

*Center for Biomedical Research, University of Texas Health Center, Tyler, TX 75708; and †Division of Immunology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215

Received for publication June 14, 2007. Accepted for publication October 21, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by research grants from Juvenile Diabetes Research Foundation International and from American Diabetes Association.

[2] Address correspondence and reprint requests to Dr. Zhenhua Dai, University of Texas Health Center, 11937 U.S. Highway 271, Tyler, TX 75708. E-mail address: zhenhua.dai@uthct.edu

[3] Abbreviations used in this paper: Treg, regulatory T cell; T_{EM}, effector memory T cell; T_{CM}, central memory T cell; WT, wild type; DLN, draining lymph nodes; MST, median survival time.

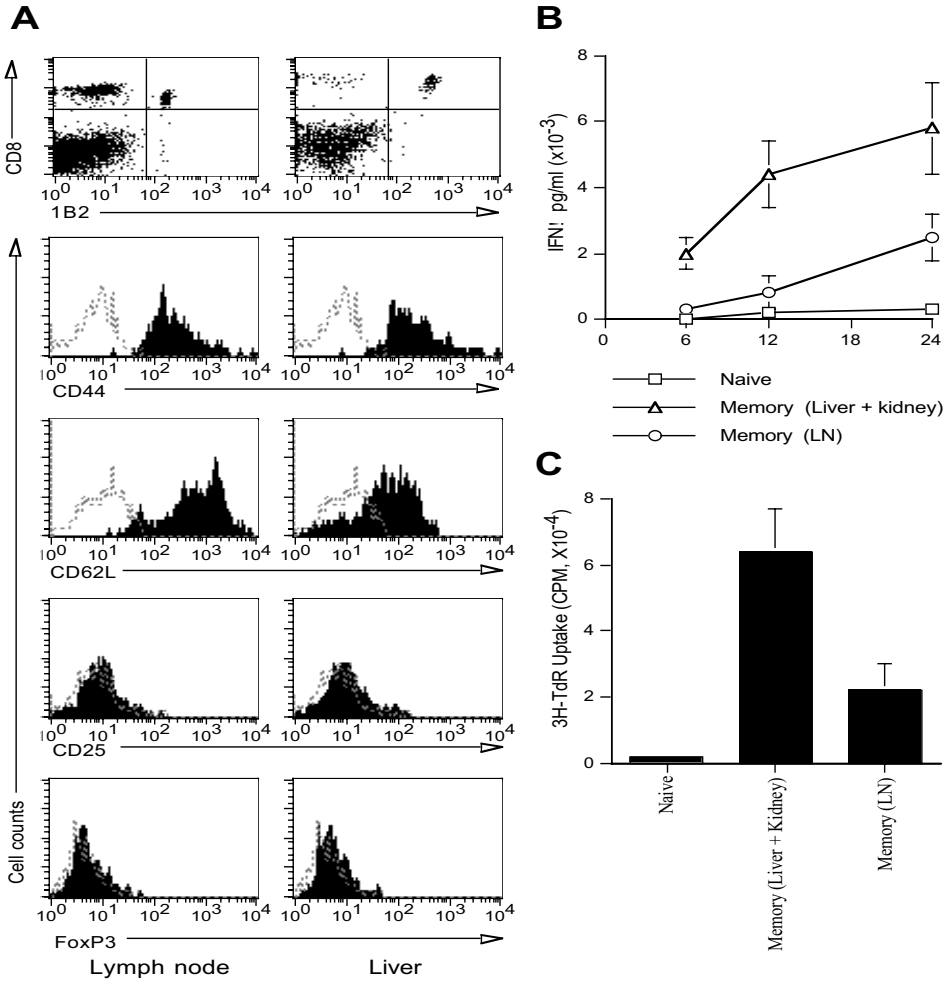

FIGURE 1. The generation of 2C memory $CD8^+CD44^{high}$ T cells. Five $\times$ 10^6 of 2C naive $CD8^+$ T cells ($CD8^+1B2^+CD25^-CD44^{low}$) were sorted out and transferred to B6 mice that were transplanted with BALB/c islets 1 day later. Ten weeks later, 2C memory cells were isolated from mesenteric lymph nodes and livers/kidneys etc., stained, and analyzed by FACS. *A*, The phenotypes of 2C memory T cells after gating on $CD8^+1B2^+$ cell population. The dotted lines represent the isotype controls. A representative from three separate experiments is shown. *B*, 2C T_{CM} cells ($CD8^+1B2^+CD44^{high}CD62L^{high}$) isolated from lymph nodes and spleens or 2C T_{EM} cells ($CD8^+1B2^+CD44^{high}CD62L^{low}$) isolated from the liver/kidney were incubated with irradiated BALB/c splenocytes in 96-well plates for up to 24 h to measure IFN-γ production by ELISA. *C*, Similar cells were cultured for 24 h to detect 2C memory T cell proliferation by [^{3}H]TdR uptakes. Data are presented as mean $\pm$ SD (n = 5–6 wells per group). One of three independent experiments is shown.

cell sorter (BD Biosciences). The 2C $CD8^+$ T cells specifically recognize L^d alloantigen on BALB/c cells and can be tracked by a specific Ab 1B2, are then referred to as $CD8^+1B2^+$. In brief, splenocytes from $2CRag^{-/-}$ mice (B6) were first incubated with anti-CD8-PE, anti-CD25-FITC, and anti-CD44-PerCP Abs (BD Pharmingen) and $CD8^+CD25^-CD44^{low}$ T cells were then sorted out by FACSAria. The purity of naive $CD8^+$ cells was typically >98%. The 2C naive $CD8^+$ T cells (5×10^6) were then adoptively transferred to B6 mice that were transplanted with BALB/c islets 1 day later. The 2C $CD8^+1B2^+$ memory cells were finally detected by staining with anti-CD8-PE, 1B2, and rat anti-mouse IgG1-FITC and enumerated by a FACSCalibur (BD Biosciences) 10 wk after transplantation. $CD8^+$ T cell memory phenotype was further confirmed by staining with anti-CD62L-allophycocanin or anti-CD44-allophycocanin (BD Pharmingen) as described previously (6). To purify memory $CD8^+$ cells for adoptive transfer experiments or in vitro studies, central memory $CD8^+$ T cells ($1B2^+CD8^+CD44^{high}CD62L^{high}$) were isolated from spleens and mesenteric LNs by FACSAria cell sorting after four-color staining, while effector memory $CD8^+$ T cells ($1B2^+CD8^+CD44^{high}CD62L^{low}$) were purified from livers and kidneys (6, 14, 36).

2C cell proliferation in vitro and supernatant IFN-γ measurement

Naive or memory 2C cells (1×10^4/well), isolated by FACS cell sorting from lymph nodes or livers and kidneys of recipient mice, were cultured with irradiated BALB/c spleen cells (1×10^4/well) in 96-well plates (Corning Costar) in complete RPMI 1640 medium (10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin). Cells were cultured for 24 h and pulsed with [^{3}H]thymidine for the last 6 h. Cells were then harvested and analyzed by a scintillation counter (PerkinElmer). To measure IFN-γ production by 2C memory cells, the same cells were cultured for up to 24 h and IFN-γ level in the supernatant was detected by a mouse cytokine ELISA kit according to the manufacturer's instructions (Invitrogen Life Technologies).

2C T_{CM} cell expansion in vitro

The 2C T_{CM} cells generated in vivo were isolated and cultured in 24-well plates in the complete RPMI 1640 medium containing 10% FCS, 2 mM glutamine, 10 mM pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin in the presence of 20 ng/ml IL-7 and IL-15 (R&D Systems) for 7 days, because IL-15 in the culture promotes effector/memory T cells to differentiate into T_{CM} cells (37, 38). The culture was refreshed by replacement with new medium containing fresh cytokines on day 4 after the culture. Cells were finally washed and transferred to recipient mice via tail vein injection.

Isolation of tissue-infiltrating cells

Tissue-infiltrating cells were isolated as described in our previous publications (6, 14). In brief, the livers and kidneys were perfused in situ with heparinized 0.9% saline. They were then minced and digested at 37°C for 30 min in 20 ml RPMI 1640 medium containing 5% FCS and 350 U/ml collagenase (Sigma-Aldrich). To clear the debris, cell suspensions were rapidly passed down 70 μm cell strainer, then mixed with Percoll solution (Sigma-Aldrich) to a concentration of 30%, and centrifuged at 2000 rpm for 15 min at room temperature. The pellet was resuspended and stained before analysis.

Analysis of T cell proliferation in vivo by BrdU labeling

Recipient mice were pulsed i.p. with 0.8 mg of BrdU (Sigma-Aldrich) 6 days after transplantation. Twenty-four h later, renal graft-infiltrating cells or renal draining lymph node cells were isolated and stained with anti-CD8-PE and 1B2, followed by rat anti-mouse IgG1-biotin and streptavidin-PerCP. Cells were then fixed in 70% ethanol followed by 1% paraformaldehyde and incubated with 50 U/ml DNase I (Sigma-Aldrich). Cells were finally stained with anti-BrdU-FITC (BD Biosciences) and analyzed by a four-color FACSCalibur (6, 14). For analyzing homeostatic proliferation,

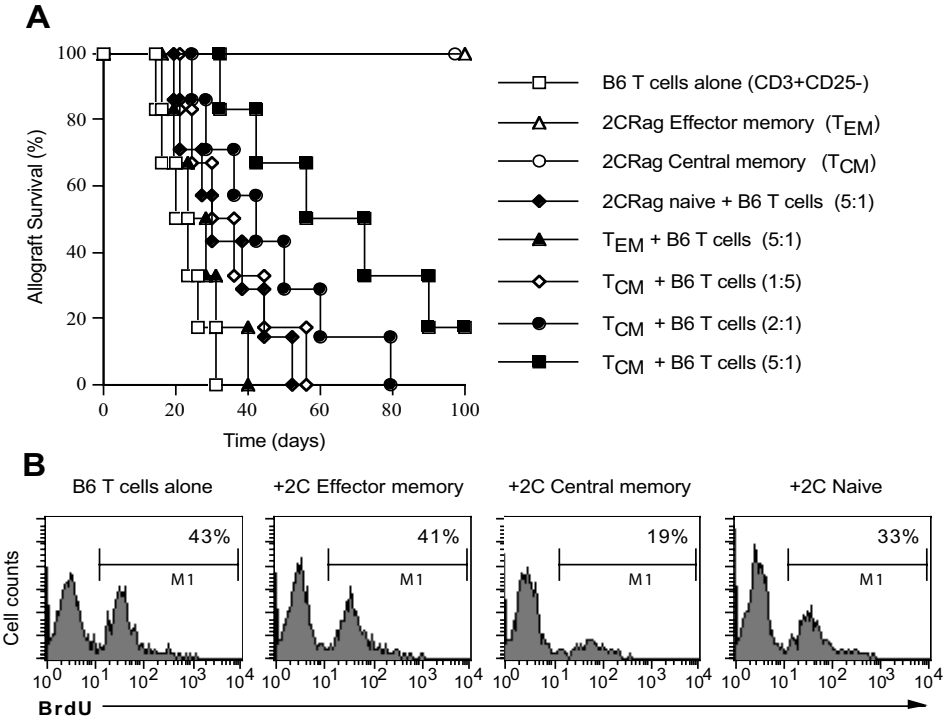

FIGURE 2. 2C T_{CM}, but not T_{EM}, cells suppress responder T cell function in vivo. One $\times$ 10^5 of naive B6 T cells alone ($CD3^+CD25^-$) (□, $n = 6$), together with 5×10^5 of 2C naive T cells ($CD8^+1B2^+CD44^{low}CD25^-$) (♦, $n = 7$), 2C T_{EM} cells (▲, $n = 6$), 2C T_{CM} cells (■, $n = 6$), with 0.2×10^5 (1:5, ◇, $n = 6$) or 2×10^5 of 2C T_{CM} cells (2:1, ●, $n = 7$) were adoptively transferred i.v. to $Rag1^{-/-}$ mice that were transplanted with islets derived from C3H/HeJ mice. As controls, 2C T_{EM} cells (△, $n = 6$) or T_{CM} cells (○, $n = 7$) alone were also transferred to $Rag1^{-/-}$ mice that received islets from C3H/HeJ mice. *A*, Islet allograft rejection was observed. *B*, Renal DLN cells were harvested 1 wk after renal subcapsular islet transplantation, and stained for B6 responder T cell proliferation by BrdU uptakes after gating on the $1B2^-CD3^+$ population. For these experiments, 2C memory or naive cells, together with responder B6 naive T cells, were transferred at the ratio of 5:1. One representative of four independent experiments is shown.

spleen cells were isolated and stained with the same method 7 days after cell transfer to $Rag1^{-/-}$ mice.

Intracellular cytokine staining

To determine intracellular TGFβ1 expression by 2C memory cells, renal draining lymph nodes (DLN) cells were first isolated and stained for surface markers with anti-CD8-PE, 1B2, and rat anti-mouse IgG1-FITC. Cells were then fixed with 2% paraformaldehyde, permeabilized in 0.5% saponin before staining with anti-TGFβ1-biotin and streptavidin-PerCP (BD Pharmingen). Cells were finally analyzed by a FACSCalibur (BD Biosciences).

Treatment of mice with IL-15 Fc, recombinant IL-15, and other Abs

To block IL-15 in vivo, recipient mice were treated with the fusion protein IL-15 Fc at 2.5 μg per mouse daily for 14 days as described previously (39). To provide additional IL-15, recipient mice were treated i.p. with 0.5 μg of recombinant mouse IL-15 (R&D Systems) on days 0, 2, 4, 6, and 8 after transplantation. To neutralize TGFβ1, recipient mice were treated with 0.1 mg of anti-TGFβ1 (R&D Systems) or isotype Ab on days 0, 2, 4, 6, and 8 after transplantation. Anti- IFN-γ Ab (R&D Systems) or isotype Ab was injected i.p. at 0.25 mg on days 0, 2, 4, 6, and 8 after transplantation (40).

Statistical analysis

The analysis of allograft survival data was performed using the Kaplan-Meier (log-rank test). Comparison of means was conducted using ANOVA.

Results

The generation of bystander or Ag-specific 2C memory T cells

To generate Ag-specific memory $CD8^+$ T cells, we used 2C transgenic $CD8^+$ T cells that specifically recognize the L^d alloantigen on BALB/c cells and can be tracked by a clonotypic Ab, 1B2, referred to as $CD8^+1B2^+$. 2C transgenic mice were backcrossed to $Rag1^{-/-}$ background and $2CRag^{-/-}$ mice have only a monoclonal T cell population that bears a single transgenic TCR. Hence, $CD8^+1B2^+$ T cells from $2CRag^{-/-}$ mice specifically recognize the L^d alloantigen. Naive $CD8^+$ T cells ($CD8^+CD25^-CD44^{low}$) from $2CRag^{-/-}$ mice were purified and transferred to WT B6 mice that were transplanted with BALB/c islets 1 day later. Ten weeks later, 2C memory $CD8^+$ T cells were isolated and analyzed by FACS. As shown in Fig. 1*A*, memory 2C cells were confirmed to have memory phenotypes because 2C cells from mesenteric lymph nodes were $CD44^{high}$ and largely $CD62L^{high}$, a central memory (T_{CM}) marker, while those from livers were $CD44^{high}$ and mostly $CD62L^{low}$, an effector memory (T_{EM}) marker (37, 41–43). Moreover, these 2C memory cells were $CD25^-FoxP3^-$, a nonregulatory cell phenotype. 2C T_{EM} cells produced significant IFN-γ in vitro at 6 h while 2C naive and T_{CM} cells did not (Fig. 1*B*). However, 2C T_{CM} cells produced significant IFN-γ at 24 h, though less than the level produced by T_{EM} cells (2.5 ± 0.7 vs 5.8 ± 1.4, $p = 0.0065$). T_{EM} cells proliferated vigorously at 24 h whereas T_{CM} cells underwent much slower expansion than T_{EM} cells (6.4 ± 1.3 vs 2.2 ± 0.7, $p < 0.005$) (Fig. 1*C*). Naive 2C cells did not proliferate at 24 h. These data further confirmed that 2C memory cells generated in this study are true memory T cells.

2C T_{CM}, but not T_{EM}, cells suppress responder T cell function in vivo

To determine whether 2C memory cells affect or interfere with naive T cell function, 2C memory cells, together with B6 naive T cells were transferred to $Rag1^{-/-}$ mice that were transplanted with

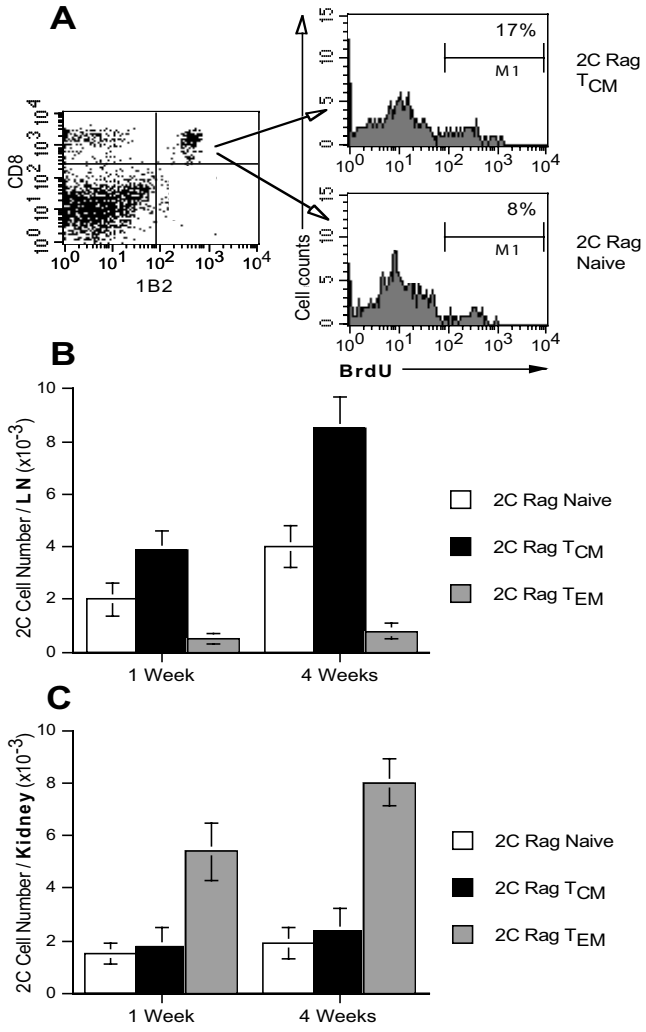

FIGURE 3. 2C T_{CM} cells migrate to the DLN and undergo faster expansion than 2C naive T cells. A total of 2.5×10^5 of naive B6 T cells ($CD3^+$ $CD25^-$) together with 5×10^5 of 2C naive, T_{CM}, or T_{EM} cells were transferred to Rag1$^{-/-}$ mice that were transplanted with C3H/HeJ islets. *A*, Renal DLN cells were harvested 1 wk after renal subcapsular islet transplantation, and stained for CD8 and 1B2, and 2C cell proliferation by BrdU uptakes. One representative of three separate experiments is shown. *B*, 2C cells isolated from the DLN were quantified by staining for CD8 and 1B2 and FACS analysis. *C*, 2C cells, isolated from the kidney containing islet allografts, were quantified by the same method. Results are shown as mean ± SD (n = 5–6 mice per group). One representative of three independent experiments is shown.

C3H/HeJ islets. As shown in Fig. 2*A*, neither 2C T_{CM} nor T_{EM} cells alone rejected C3H/HeJ islet grafts while transfer of WT B6 naive T cells did so, demonstrating that Ag-specific, lineage-differentiated 2C memory cells do not respond to an irrelevant Ag. The transfer of 2C T_{CM} cells, at the ratio of 1:5 or 2:1 to B6 naive T cells, modestly but significantly suppressed allograft rejection mediated by B6 naive T cells (median survival time, MST = 33 vs 22 or 42 vs 22 days, both $p < 0.05$) while transfer of 2C T_{CM} cells at the ratio of 5:1 further suppressed allograft rejection mediated by B6 naive T cells (MST = 64 vs 22 days, $p < 0.005$). Nevertheless, transfer of 2C T_{EM} cells even at the ratio of 5:1 did not (MST = 25 vs 22 days, $p = 0.1467$), suggesting that the transfer of T_{CM}, but not T_{EM} cells, suppresses responder T cell function. Transfer of 2C naive cells slightly delayed the allograft rejection (MST = 29 vs 22 days, $p = 0.0389$). Therefore, 2C T_{CM} cells were much more potent in the suppression of allograft rejection than 2C naive T cells (MST = 64 vs 29 days, $p = 0.0075$), suggesting that 2C T_{CM} cells have an advantage over 2C naive T cells in their suppression.

Rag1$^{-/-}$ recipients that received 2C memory cells and/or B6 naive T cells as well as islet allografts were sacrificed to measure B6 T cell proliferation in renal DLN by BrdU uptakes 1 wk after renal subcapsular islet transplantation. As shown in Fig. 2*B*, transfer of 2C T_{EM} cells did not suppress responder T cell proliferation (BrdU-positive: 41 ± 2% vs 43 ± 3%, $p > 0.05$) while transfer of T_{CM} cells did so (19 ± 1% vs 43 ± 3%, $p < 0.05$). Moreover, 2C T_{CM} cells were more potent in the suppression of responder B6 T cell proliferation than 2C naive T cells (19 ± 1% vs 33 ± 2%, $p < 0.05$). Taken together, these data suggest that bystander T_{CM} cells suppress allograft rejection by limiting T cell expansion/activation. It is noteworthy that 2C T_{CM} cells do not suppress naive T cell proliferation in vitro (data not shown).

2C T_{CM} cells can migrate to the DLN and undergo faster expansion than 2C naive T cells after islet transplantation

To investigate why T_{CM}, but not T_{EM}, cells suppress allograft rejection, transplanted Rag1$^{-/-}$ mice that received 2C memory cells and B6 naive T cells were sacrificed 1 wk after transplantation. Recipient mice were pulsed i.p. with BrdU for 24 h and BrdU uptakes by 2C cells in renal DLN were measured. As shown in Fig. 3*A*, 2C T_{CM} cells in the DLN proliferated at a faster pace than 2C naive T cells (BrdU-positive: 17 ± 2% vs 8 ± 1%, $p < 0.05$). This finding may partially interpret why 2C T_{CM} cells are more potent in suppression than 2C naive T cells, because T_{CM} cells exhibit quicker turnover and are hence more effective in suppression than

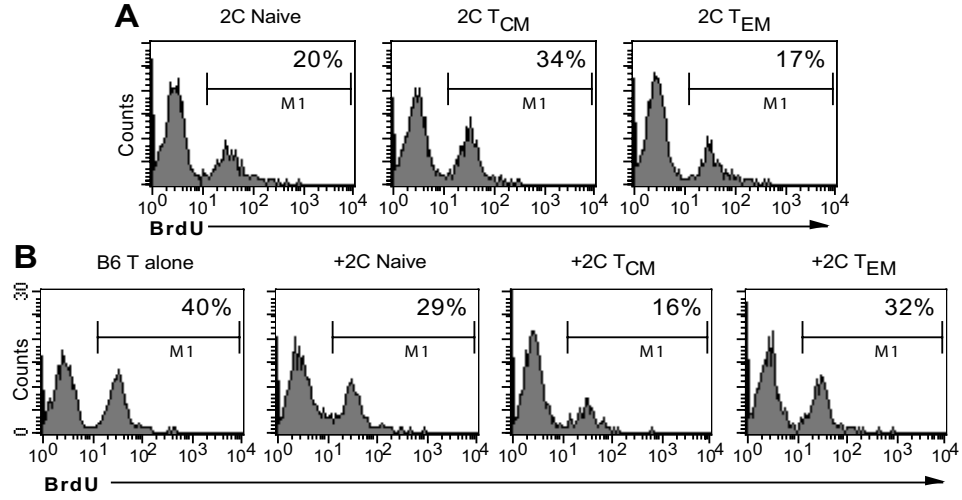

FIGURE 4. Homeostatic proliferation of 2C memory cells and nontransgenic B6 T cells. Naive B6 T cells alone or together with 2C naive, 2C T_{CM}, or T_{EM} cells were transferred to Rag1$^{-/-}$ mice. One week later, spleen cells of recipients were isolated, stained, and analyzed for their proliferation by BrdU uptakes. *A*, Percentage of BrdU-positive 2C naive and memory cells after gating on $1B2^+$ 2C population. *B*, Percentage of BrdU-positive B6 T cells after gating on $CD3^+1B2^-$ non-2C T cells. One of three separate experiments is shown.

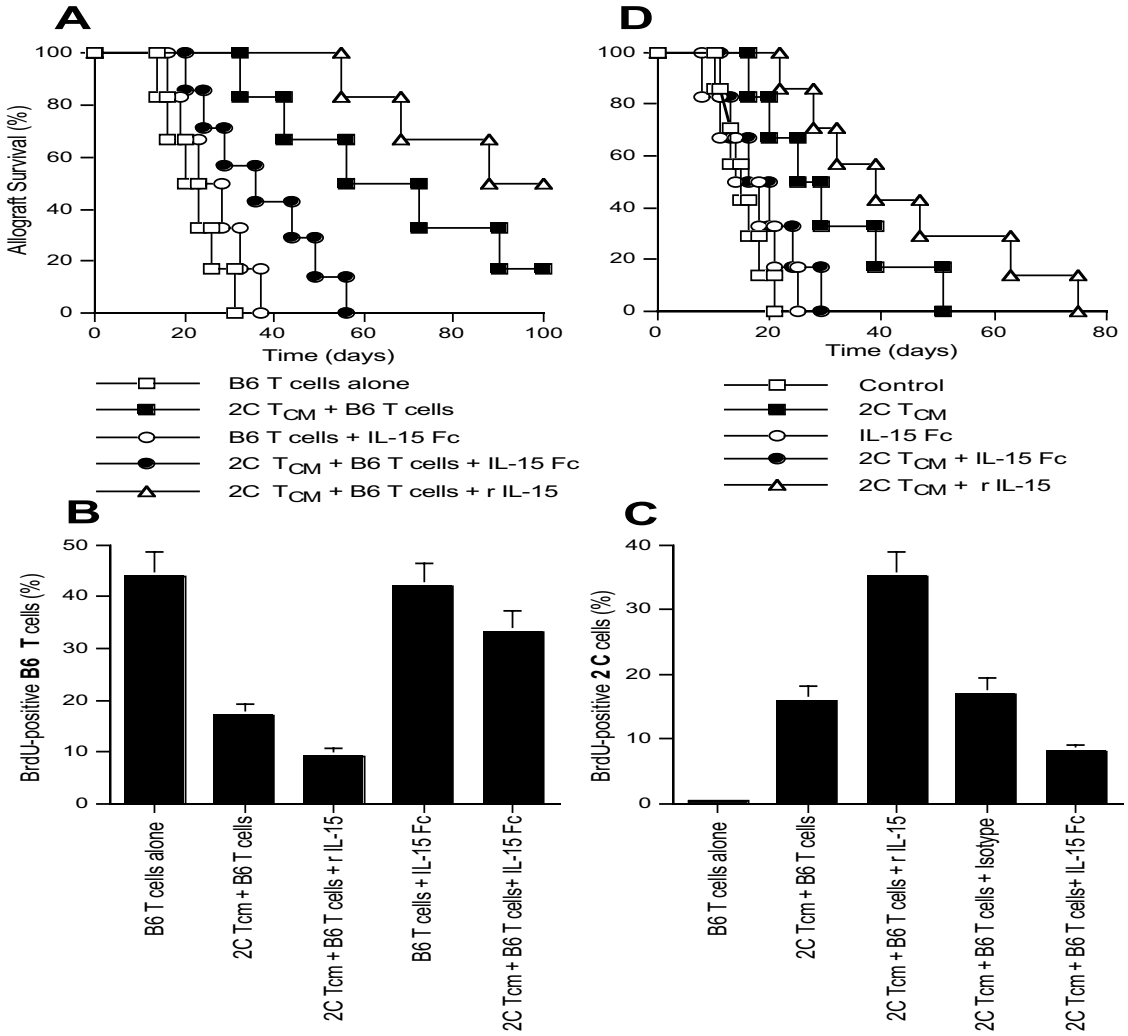

FIGURE 5. IL-15 is required for T_{CM}-mediated suppression of allograft rejection and the suppression of responder T cell proliferation. *A*, $Rag1^{-/-}$ mice were transplanted with C3H/HeJ islets and received i.v. 1×10^5 of naive B6 T cells alone ($CD3^+CD25^-$) (□, $n = 6$), B6 T cells plus IL-15 Fc (○, $n = 6$), 1×10^5 of B6 T cells together with 5×10^5 of 2C T_{CM} cells (■, $n = 6$), with 5×10^5 of 2C T_{CM} cells plus IL-15 Fc (●, $n = 7$), or with 5×10^5 of 2C T_{CM} cells plus mouse rIL-15 (△, $n = 6$). Islet allograft rejection was observed. *B*, Responder B6 T cells derived from the renal DLN were stained with anti-BrdU and analyzed by FACS. BrdU-positive cells were gated on $CD3^+1B2^-$ population. *C*, 2C cells derived from the renal DLN were stained with anti-BrdU and analyzed by the same method. BrdU-positive cells were gated on $CD8^+1B2^+$ population. Results are shown as mean ± SD of the percentage of BrdU-positive cells ($n = 5$–6 mice per group). One representative of three independent experiments is shown. *D*, WT B6 recipient mice were transplanted with C3H/HeJ islets (□, $n = 7$) under the kidney capsule. Some recipients were injected with 5×10^5 of 2C T_{CM} cells alone (■, $n = 6$) under the kidney capsule at the same time when islets were injected, with IL-15 Fc alone (○, $n = 6$), 2C T_{CM} cells plus IL-15 Fc (●, $n = 6$), or 2C T_{CM} cells plus rIL-15 (△, $n = 7$). Islet allograft rejection was observed.

naive T cells. Very fewer 2C cells were detected in the DLN when 2C T_{EM} cells were transferred (Fig. 3*B*). The absolute count of 2C T_{CM} cells was much higher in the DLN than 2C naive T cell count (1 wk: 3.9 ± 0.7 vs 2.0 ± 0.6, $p = 0.0088$ and 4 wk: 8.5 ± 1.2 vs 4.0 ± 0.8, $p = 0.0076$) (Fig. 3*B*). Moreover, as shown in Fig. 3*C*, there were more 2C cells infiltrating the kidney when 2C T_{EM} cells were transferred than when 2C T_{CM} cells were (1 wk: 5.4 ± 1.1 vs 1.8 ± 0.7, $p < 0.005$; 4 wk: 2.4 ± 0.7 vs 8.1 ± 0.8, $p < 0.005$). These data suggest that allospecific T_{EM} cells incline to stay in tissues while T_{CM} cells are mainly harbored in the LNs.

2C T_{CM} cells undergo faster homeostatic proliferation and are more potent in suppression of B6 T cell homeostatic proliferation than their naive and T_{EM} counterparts

To measure the homeostatic proliferation of 2C memory cells and their capability of inhibiting B6 T cell expansion, naive B6 T cells alone, or together with 2C naive, 2C T_{CM} or T_{EM} cells were transferred to $Rag1^{-/-}$ mice. One week later, spleen cells of recipients were isolated, stained, and analyzed for their proliferation by BrdU uptakes. As shown in Fig. 4*A*, 2C T_{CM} cells underwent faster homeostatic proliferation than 2C naive and 2C T_{EM} cells (BrdU-positive: 34 ± 3% vs 20 ± 3 or 17 ± 2, both $p < 0.05$). Moreover, 2C T_{CM} cells were much more potent in suppression of B6 T cell proliferation than their naive and T_{EM} counterparts (BrdU-positive: 16 ± 1% vs 29 ± 2 or 32 ± 3, both $p < 0.05$) (Fig. 4*B*).

IL-15 is required for T_{CM}-mediated suppression of allograft rejection as well as responder T cell proliferation

IL-15 selectively stimulates memory $CD8^+$ T cell proliferation and is required for their generation (44, 45). In this study, we tested whether IL-15 is required for T_{CM}-mediated suppression. 2C T_{CM} cells, together with B6 naive T cells, were transferred to $Rag1^{-/-}$ mice that received C3H/HeJ islets. Some recipients were treated with rIL-15 or 1L-15-blocking protein, IL-15 Fc. As shown in

Fig. 5*A*, IL-15 Fc alone did not suppress allograft rejection mediated by B6 naive T cells (MST = 25 vs 22 days, $p = 0.1356$). Instead, IL-15 Fc largely alleviated the suppression of allograft rejection by 2C T_{CM} cells (MST = 35 vs 64 days, $p = 0.0104$). The isotype protein did not affect allograft survival (data not shown). In contrast, administering rIL-15 enhanced T_{CM}-mediated suppression of allograft rejection (MST = 98 vs 64 days, $p = 0.007$). These data demonstrate that the suppression of allograft rejection by 2C T_{CM} cells is largely dependent on IL-15.

To study the effect of IL-15 on the proliferation of 2C T_{CM} cells as well as responder T cells, DLN cells, derived from transplanted $Rag1^{-/-}$ mice that received both cells, were stained for BrdU and analyzed 1 wk after transplantation. As shown in Fig. 5*B*, 2C T_{CM} cells suppressed responder B6 T cell proliferation in DLN (BrdU-positive: 19 ± 2.6 vs 44 ± 4.7, $p = 0.0035$). Administering rIL-15 further exacerbated the suppression (9 ± 1.8 vs 19 ± 2.6, $p = 0.0046$), whereas IL-15 Fc largely abolished T_{CM}-mediated suppression (34 ± 4.2 vs 19 ± 2.6, $p = 0.0078$), suggesting that IL-15 is required for the suppression of responder T cell proliferation by bystander T_{CM} cells. However, IL-15 Fc did not affect the proliferation of responder T cells themselves. In contrast, as shown in Fig. 5*C*, rIL-15 promoted the proliferation of 2C T_{CM} cells (BrdU-positive: 35 ± 3.9 vs 17 ± 2.1, $p < 0.005$) while IL-15 Fc suppressed their proliferation (8 ± 1.2 vs 17 ± 2.1, $p = 0.0059$), indicating that IL-15 is critical for 2C T_{CM} cell turnover. Taken together, IL-15 is required for the expansion of bystander T_{CM} cells and their suppression of responder T cell proliferation.

The suppression of allograft rejection by bystander T_{CM} cells in WT mice

To determine whether T_{CM} cells suppress allograft rejection in physiologic hosts, WT B6 mice were injected with both islet allografts and 2C T_{CM} cells under the same kidney capsule at the same time, because systemic administration of Treg cells is insufficient to prolong allograft survival in WT animals. As shown in Fig. 5*D*, T_{CM} cells indeed inhibited allograft rejection (MST = 27 vs 15 days, $p = 0.0084$). IL-15 Fc alone did not affect allograft survival (MST = 16 vs 15 days, $p > 0.05$), whereas IL-15 Fc largely abolished the suppression of allograft rejection by T_{CM} cells (MST = 17 vs 27 days, $p = 0.0105$). Moreover, rIL-15 enhanced T_{CM}-mediated suppression (MST = 40 vs 27 days, $p = 0.0121$). Local administration of 2C T_{EM} cells under the kidney capsule did not prolong allograft survival (data not shown). These findings suggest that bystander T_{CM} cells, when administered locally, can suppress allograft rejection even in normal animals and this suppression requires the presence of IL-15. However, T_{CM}-mediated suppression in WT mice appears to be limited, though significant, as all allografts were eventually rejected, indicating that regulation alone is insufficient to induce long-term allograft survival in immune competent WT mice or that the transfer of more bystander T_{CM} cells may be needed for longer suppression of allograft rejection in WT mice. But it is not feasible to generate a large number of T_{CM} cells in vivo.

Transforming growth factor β1 is required for the suppression of allograft rejection by bystander T_{CM} cells

TGFβ1 is a predominant TGFβ isoform in lymphoid organs. It is one of the major immunosuppressive cytokines (46, 47). To ask whether TGFβ1 plays a role in T_{CM}-mediated suppression, WT B6 mice were injected with both islet allografts and 2C T_{CM} cells under the same kidney capsule and treated with anti-TGFβ1 Ab. As shown in Fig. 6*A*, anti-TGFβ1 treatment alone did not significantly alter allograft survival (MST = 13 vs 15 days, $p > 0.05$), whereas anti-TGFβ1 treatment shortened allograft survival induced by the transfer of 2C T_{CM} cells (MST = 16 vs 27 days, $p = 0.013$). The isotype Ab treatment did not affect allograft survival induced by 2C T_{CM} cells (MST = 28 vs 27 days, $p > 0.05$). These findings suggest that the suppression of allograft rejection by bystander 2C T_{CM} cells is dependent on the presence of TGFβ1. To further determine whether 2C T_{CM} cells actually express TGFβ1, renal DLN cells, derived from recipients that received both islet allografts and 2C cells under the kidney capsule, were stained and analyzed for intracellular TGFβ1 expression by 2C cells. As shown in Fig. 6*B*, a sizable portion of 2C cells expressed TGFβ1 when either 2C T_{CM} or T_{EM} cells were transferred, while 2C naive cells transferred did not express a significant amount of TGFβ1, implying that TGFβ1 may be involved in the suppression by bystander 2C T_{CM} cells. Interestingly, 2C T_{EM} cells also expressed a significant amount of TGFβ1, albeit slightly less than T_{CM} cells (30 vs 36%). These findings suggest that TGFβ1 is required for T_{CM} cell-mediated suppression but not sufficient for T_{EM} cells to

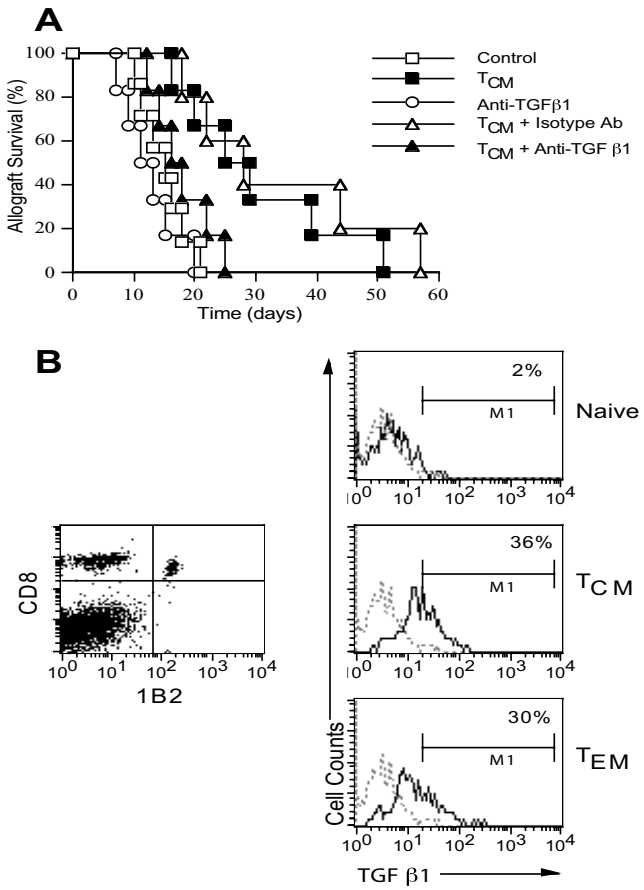

FIGURE 6. TGFβ1 is essential for bystander T_{CM}-mediated suppression of allograft rejection. *A*, WT B6 recipient mice were transplanted with C3H/HeJ islets (□, $n = 7$) under the kidney capsule. Some recipients were injected with 5×10^5 of 2C T_{CM} cells alone (■, $n = 6$) under the kidney capsule at the same time when islets were injected, with anti-TGFβ1 alone (○, $n = 6$), 2C T_{CM} cells plus anti-TGFβ1 (▲, $n = 6$), or 2C T_{CM} cells plus isotype Ab (△, $n = 5$). Islet allograft rejection was observed. *B*, WT B6 mice were transplanted with C3H/HeJ islets under the kidney capsule and received 5×10^5 of 2C naive, T_{CM}, or T_{EM} cells via the same location. One week later, cells from renal DLN were isolated and stained for surface markers CD8 and 1B2 as well as intracellular TGFβ1. Because very fewer 2C T_{EM} cells stayed in renal DLN, they were isolated from the kidney tissue and also stained for intracellular TGFβ1 for the comparison. Histograms are shown after gating on $CD8^+1B2^+$ populations. The dotted lines represent the isotype control. One representative of three independent experiments is shown.

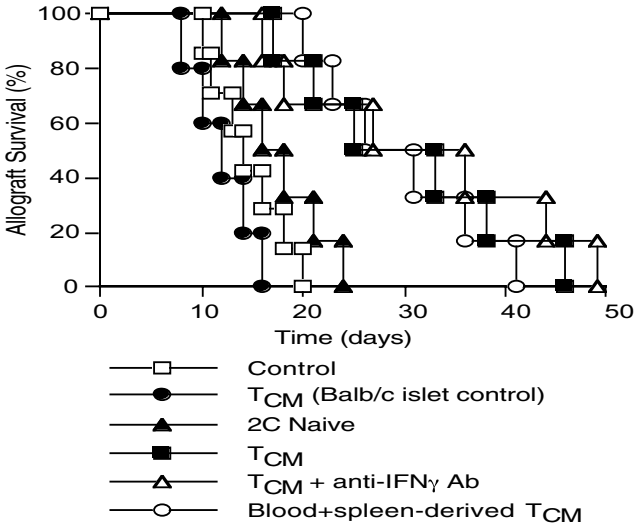

FIGURE 7. Intravenous administration of in vitro-expanded 2C T_{CM} but not 2C naive cells suppresses allograft rejection in WT mice. 2C T_{CM} cells isolated from spleens and mesenteric lymph nodes were isolated and cultured in 24-well plates in the complete RPMI 1640 medium containing 10% FCS in the presence of IL-7 (20 ng/ml) and IL-15 (20 ng/ml) for 7 days. WT B6 recipients were transplanted with C3H/HeJ islets alone (□, $n = 7$) or received 5×10^6 of cultured 2C T_{CM} cells (■, $n = 6$) or 5×10^6 of 2C naive cells (▲, $n = 6$) via tail vein on both day −1 and 5 after transplantation. Some recipients were treated with neutralizing anti-IFN-γ Ab after 2C T_{CM} cell transfer (△, $n = 6$). 2C T_{CM} cells do not react to an allograft from C3H/HeJ mice and hence are bystanders. As a control, some recipients were transplanted with BALB/C islets (●, $n = 5$) to ensure that 2C T_{CM} cells respond to a BALB/C donor and do not become universal suppressors after culture. In another group, 2C T_{CM} cells isolated from the blood and spleens were cultured in the same conditions and transferred to islet recipients (○, $n = 6$). Islet allograft rejection was observed.

exert their suppression. Perhaps TGFβ1-positive T_{EM} cells are unable to exert their suppression due to their inability to largely migrate to lymph nodes.

When injected i.v. with a large number, in vitro-expanded 2C T_{CM} cells can suppress allograft rejection in WT mice

So far our data have shown that administering a small number of T_{CM} cells (5×10^5), generated directly in vivo, can significantly prolong allograft survival via either i.v. injection in immune-deficient $Rag1^{-/-}$ mice or local injection in immune-competent WT mice. To further determine whether i.v. administration of bystander T_{CM} cells suppresses allograft rejection in WT mice, we expanded T_{CM} cells in vitro and transferred a large number of these cells (5×10^6/per dose for two doses) i.v. back to WT B6 recipient mice that received C3H/HeJ islets. As shown in Fig. 7, systemic use of a large number of in vitro-expanded 2C T_{CM} cells, derived from spleens plus LNs, indeed suppressed islet allograft rejection (MST = 29 vs 15 days, $p < 0.05$), whereas systemic administration of the same number of 2C naive cells did not (MST = 17 vs 15 days, $p > 0.05$). Interestingly, neutralizing IFN-γ did not abolish the suppression mediated by 2C T_{CM} cells (MST = 31 vs 15 days, $p < 0.05$). Moreover, 2C T_{CM} cells derived from the blood plus spleens were capable of suppressing allograft rejection (MST = 28 vs 15 days, $p < 0.05$), suggesting that bystander T_{CM} cells derived from non-mesenteric lymph nodes organs are also suppressive. 2C T_{EM} cell transfer experiments were not done because we were unable to get enough cell numbers because of their inability to expand greatly in vitro. As a control, the same number of 2C T_{CM} cells did not suppress the rejection of BALB/C islet allografts (MST = 13 vs15 days, $p > 0.05$), suggesting that 2C T_{CM} cells are not universally suppressive after the culture. In vitro-expanded T_{CM} cells remained FoxP3 negative (data not shown).

Discussion

Using allograft rejection as readout and Ag-specific monoclonal memory T cells as bystanders, we investigated the role of bystander $CD8^+$ T_{CM} cells in an alloimmune response. We found that T_{CM}, but not T_{EM}, $CD8^+$ T cells suppress allograft rejection and T cell proliferation in vivo. Both T_{CM} and naive T cells migrated to DLN, whereas T_{CM} cells exhibited faster turnover than their naive counterparts. However, T_{EM} cells migrated to inflammatory sites, but not DLN, and yet failed to exert suppression. Moreover, bystander T_{CM}-mediated suppression was largely dependent on IL-15 and TGFβ1, as IL-15 was required for the homeostasis of bystander T_{CM} cells while neutralizing TGFβ1 abolished the suppression of allograft rejection. Thus, bystander T_{CM}, but not T_{EM}, $CD8^+$ cells are potent suppressors rather than true bystanders. This study reveals an unexpected role of bystander memory T cells in adaptive immunity and may have an impact on cellular immunology.

So far studies using memory T cells generated in vivo as a broad therapeutic approach are hampered due to the limited number of memory T cells retrieved directly from animals. These memory T cells in a small number are less effective when administered systemically in a WT animal. Our model has taken advantage of alloAg-specific monoclonal transgenic memory T cells. In particular, these cells are directly introduced under the kidney capsule when islet grafts are transplanted in the same location. They are therefore able to exert locally optimal suppression around islet grafts with a relatively small cell number. This finding suggests that bystander memory cell therapies are particularly useful for inducing long-term survival of cellular transplants including islets that has the potential to cure type 1 diabetes. In contrast, we find that a large number of in vitro-expanded T_{CM} cells, when administered i.v., are also capable of suppressing islet allograft rejection. The systemic administration of T_{CM} cells will dramatically broaden their applicability to other types of solid organ transplants.

It is unclear whether nontransgenic polyclonal bystander memory T cells derived from WT mice can also suppress allograft rejection. Unfortunately, with current techniques, one cannot separate nontransgenic bystander memory T cells from Ag-specific ones in WT animals. Polyclonal bystander memory T cells derived from immunized WT animals would still contain a very small portion of alloreactive T cells that are able to reject a third party allograft by themselves. Nevertheless, manipulating or engineering monoclonal bystander memory T cells in humans as well as rodents are feasible and may represent a new therapeutic strategy for inducing long-term allograft survival.

In this study, we demonstrated that 2C T_{EM} cells prefer to migrate to the inflammatory site, but fail to exert their suppression, while 2C T_{CM} cells migrate to DLN and do suppress allograft rejection. These findings suggest that the migration of bystander 2C memory cells to the DLN is required for their suppression. 2C memory cells in the DLN remained FoxP3-negative 1 wk after adoptive transfer of T_{CM} cells (data not shown), suggesting that these 2C memory cells do not develop into conventional $FoxP3^+$ Treg cells. It remains to be elucidated why bystander T_{EM} cells fail to suppress allograft rejection even though they can express TGFβ1 and migrate to the inflammatory site. We speculate that 2C memory cells need to migrate to DLN to suppress responder T cell activation. 2C T_{EM} cells transferred may spread all over the body and are hence diluted in the nonlymphoid tissues, while 2C T_{CM} cells are

concentrated in lymph nodes, especially renal DLN after injection into the renal capsule, to exert their suppression. Interestingly, a recent study by others has shown that tumor-reactive T_{CM} $CD8^+$ cells are superior to T_{EM} $CD8^+$ cells for antitumor immunity because their homing to secondary lymphoid tissues is required for optimal anti-tumor activity (38). Recent studies have also shown that $CD62L^{high}$ component of $CD4^+CD25^+$ Treg cells is more potent suppressors (48) and that lymph node occupancy is required for the peripheral development of Ag-specific $FoxP3^+$ Treg cells (49), indicating that the suppression of allograft rejection by either professional $FoxP3^+$ Treg or nonprofessional bystander T_{CM} cells requires lymph node occupancy.

Another possible mechanism underlying suppression mediated by bystander T_{CM} cells is their faster homeostatic proliferation than their naive and T_{EM} counterparts (Fig. 4). Their fast homeostatic proliferation suppresses responder T cell expansion and therefore delays allograft rejection while naive and T_{EM} cells proliferate slowly and do not slow down the rejection. Studies by others have also shown that homeostatic proliferation of T cells generates regulatory phenomenon (50), suggesting that competition for the niches/spaces affects not only homeostatic proliferation but also Ag-specific immune responses. In this study, however, it is likely that both homeostatic regulation and TGFβ1 are involved in the suppression mediated by bystander T_{CM} cells. Regardless of their suppressive mechanisms, our finding identifies T_{CM}, but not T_{EM}, cells as a candidate for bystander suppression and may have clinical implications for tolerance induction. It remains to be defined whether T_{CM} cells also exert their suppression by killing donor- or recipient-derived APCs, as a recent study has shown that memory $CD8^+$ T cells can kill host dendritic cells (51). We speculate that this is not the case, since $CD8^+$ T_{EM} cells, which express higher perforin and granzyme B than $CD8^+$ T_{CM} cells (52), do not suppress allograft rejection.

In conclusion, bystander T_{CM}, but not T_{EM}, $CD8^+$ T cells suppress responder T cell function in vivo in a TGFβ1-dependent manner. Their advantage in homeostatic proliferation over their naive and T_{EM} counterparts also likely plays a role in their suppression. This study answers an important fundamental question in immunology and has some important implications. For instance, nonselectively targeting memory T cells for tolerance induction may result in some side effects, because bystander T_{CM} cells regulate immunity. Although a specific immune response may be inhibited by suppressing Ag-specific memory T cell recall, a nonselective blockade of memory T cells bearing multiple TCR repertoires would abolish the suppression mediated by T_{CM} cells and therefore tip the immunologic balance. Moreover, this study may help explain why an individual's immune defense system weakens with aging, although the proportion of T cells with memory phenotypes ($CD44^{high}$) increases, because an enlarged and differentiated T_{CM} cell pool in an aged individual suppresses T cell responsiveness to various new Ags.

Disclosures

The authors have no financial conflict of interest.

References

1. Dutton, R. W., L. M. Bradley, and S. L. Swain. 1998. T cell memory. *Annu. Rev. Immunol.* 16: 201–223.
2. Sprent, J., and C. D. Surh. 2002. T cell memory. *Annu. Rev. Immunol.* 20: 551–579.
3. Ahmed, R., and D. Gray. 1996. Immunological memory and protective immunity: understanding their relation. *Science* 272: 54–60.
4. Zhai, Y., L. Meng, F. Gao, R. W. Busuttil, and J. W. Kupiec-Weglinski. 2002. Allograft rejection by primed/memory $CD8^+$ T cells is CD154 blockade resistant: therapeutic implications for sensitized transplant recipients. *J. Immunol.* 169: 4667–4673.
5. Chen, Y., P. S. Heeger, and A. Valujskikh. 2004. In vivo helper functions of alloreactive memory $CD4^+$ T cells remain intact despite donor-specific transfusion and anti-CD40 ligand therapy. *J. Immunol.* 172: 5456–5466.
6. Dai, Z., Q. Li, Y. Wang, G. Gao, L. S. Diggs, G. Tellides, and F. G. Lakkis. 2004. $CD4^+CD25^+$ regulatory T cells suppress allograft rejection mediated by memory $CD8^+$ T cells via a CD30-dependent mechanism. *J. Clin. Invest.* 113: 310–317.
7. Trambley, J., A. W. Bingaman, A. Lin, E. T. Elwood, S.-Y. Waitze, J. Ha, M. M. Durham, M. Corbascio, S. R. Cowan, T. C. Pearson, and C. P. Larsen. 1999. Asialo $GM1^+$ $CD8^+$ T cells play a critical role in costimulation blockade-resistant allograft rejection. *J. Clin. Invest.* 104: 1715–1722.
8. Shiao, S. L., J. M. McNiff, and J. S. Pober. 2005. Memory T cells and their costimulators in human allograft injury. *J. Immunol.* 175: 4886–4896.
9. Vu, M. D., M. R. Clarkson, H. Yagita, L. A. Turka, M. H. Sayegh, and X. C. Li. 2006. Critical, but conditional, role of OX40 in memory T cell-mediated rejection. *J. Immunol.* 176: 1394–1401.
10. Chalasani, G., Z. Dai, B. Konieczny, F. K. Baddoura, and F. G. Lakkis. 2002. Recall and propagation of allospecific memory T cells independent of secondary lymphoid organs. *Proc. Natl. Acad. Sci. USA* 99: 6175–6180.
11. Obhrai, J. S., M. H. Oberbarnscheidt, T. W. Hand, L. Diggs, G. Chalasani, and F. G. Lakkis. 2006. Effector T cell differentiation and memory T cell maintenance outside secondary lymphoid organs. *J. Immunol.* 176: 4051–4058.
12. Heeger, P. S., N. S. Greenspan, S. Kuhlenschmidt, C. Dejelo, D. E. Hricik, J.A. Schulak, and M. Tary-Lehmann. 1999. Pretransplant frequency of donor-specific, IFN-γ-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J. Immunol.* 163: 2267–2275.
13. Wu, Z., S. J. Bensinger, J. Zhang, C. Chen, X. Yuan, X. Huang, J. F. Markmann, A. Kassaee, B. R. Rosengard, W. W. Hancock, et al. 2003. Homeostatic proliferation is a barrier to transplantation tolerance. *Nat. Med.* 10: 87–92.
14. Nasr, I. W., Y. Wang, G. Gao, S. Deng, L. Diggs, D. M. Rothstein, G. Tellides, F. G. Lakkis, and Z. Dai. 2005. Testicular immune privilege promotes transplantation tolerance by altering the balance between memory and regulatory T cells. *J. Immunol.* 174: 6161–6168.
15. Zhang, Y., G. Joe, E. Hexner, J. Zhu, and S. G. Emerson. 2005. Alloreactive memory T cells are responsible for the persistence of graft-versus-host disease. *J. Immunol.* 174: 3051–3058.
16. Lakkis, F. G., and M. H. Sayegh. 2003. Memory T cells: a hurdle to immunologic tolerance. *J. Am. Soc. Nephrol.* 14: 2402–2410.
17. Zhang, Q., Y. Chen, R. L. Fairchild, P. S. Heeger, and A. Valujskikh. 2006. Lymphoid sequestration of alloreactive memory CD4 T cells promotes cardiac allograft survival. *J. Immunol.* 176: 770–777.
18. Jones, N. D., M. Carvalho-Gaspar, S. Luo, M. O. Brook, L. Martin, and K. J. Wood. 2006. Effector and memory $CD8^+$ T cells can be generated in response to alloantigen independently of $CD4^+$ T cell help. *J. Immunol.* 176: 2316–2323.
19. Neujahr, D. C., C. Chen, X. Huang, J. F. Markmann, S. Cobbold, H. Waldmann, M. H. Sayegh, W. W. Hancock, and L. A. Turka. 2006. Accelerated memory cell homeostasis during T cell depletion and approaches to overcome it. *J. Immunol.* 176: 4632–4639.
20. Northrop, J. K., R. M. Thomas, A. D. Wells, and H. Shen. 2006. Epigenetic remodeling of the IL-2 and IFN-γ loci in memory CD8 T cells is influenced by CD4 T cells. *J. Immunol.* 177: 1062–1069.
21. Sakaguchi, S. 2000. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101: 455–458.
22. Shevach, E. M. 2002. $CD4^+CD25^+$ suppressor T cells: more questions than answers. *Nat. Rev. Immunol.* 2: 389.
23. Edinger, M., P. Hoffmann, J. Ermann, K. Drago, C. G. Fathman, S. Strober, and R. S. Negrin. 2003. $CD4^+CD25^+$ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat. Med.* 9: 1144–1150.
24. Zeiser, R., V. H. Nguyen, J. Z. Hou, A. Beilhack, E. Zambricki, M. Buess, C. H. Contag, and R. S. Negrin. 2007. Early CD30 signaling is critical for adoptively transferred $CD4^+CD25^+$ regulatory T cells in prevention of acute graft-versus-host disease. *Blood* 109: 2225–2233.
25. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunological self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25)-breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155: 1151–1164.
26. Suri-Payer, E., A. Amar, A. Thornton, and E. Shevach. 1998. $CD4^+CD25^+$ T cells inhibit both induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J. Immunol.* 160: 1212–1218.
27. Qin, S., S. Cobbold, H. Pope, J. Elliott, D. Kioussis, J. Davies, and H. Waldmann. 1993. "Infectious" transplantation tolerance. *Science* 259: 974–977.
28. Kingsley, C. I., M. Karim, A. R. Bushell, and K. J. Wood. 2002. $CD25^+CD4^+$ regulatory T cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses. *J. Immunol.* 168: 1080–1086.
29. Zhang, Z. X., L. Yang, K. J. Young, B. DuTemple, and L. Zhang. 2000. Identification of a previously unknown Ag-specific regulatory T cells and its mechanism of suppression. *Nat. Med.* 6: 782–789.
30. Read, S., V. Malmstrom, and F. Powrie. 2000. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. *J. Exp. Med.* 192: 295–302.
31. Lee, I., L. Wang, A. D. Wells, M. E. Dorf, E. Ozkaynak, and W. W. Hancock. 2005. Recruitment of $Foxp3^+$ T regulatory cells mediating allograft tolerance depends on the CCR4 chemokine receptor. *J. Exp. Med.* 201: 1037–1044.

32. Golshayan, D., S. Jiang, J. Tsang, M. I. Garin, C. Mottet, and R. I. Lechler. 2007. In vitro-expanded donor alloAg-specific $CD4^+CD25^+$ regulatory T cells promote experimental transplantation tolerance. *Blood* 109: 827–835.
33. Fontenot, J. D., M. A. Gavin, and A. Y. Rudensky. 2003. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol.* 4: 330–336.
34. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057–1061.
35. Bourgeois, C., and B. Stockinger. 2006. $CD25^+CD4^+$ regulatory T cells and memory T cells prevent lymphopenia-induced proliferation of naive T cells in transient states of lymphopenia. *J. Immunol.* 177: 4558–4566.
36. Liu, Z. W., H. Dai, N. Wan, T. Wang, S. Bertera, M. Trucco, and Z. Dai. 2007. Suppression of memory CD8 T cell generation and function by tryptophan catabolism. *J. Immunol.* 178: 4260–4266.
37. Weninger, W., M. A. Crowley, N. Manjunath, and U. H. von Andrian. 2001. Migratory properties of naive, effector, and memory $CD8^+$ T cells. *J. Exp. Med.* 194: 953–966.
38. Klebanoff, C. A., L. Gattinoni, P. Torabi-Parizi, K. Kerstann, A. R. Cardones, S. E. Finkelstein, D. C. Palmer, P. A. Antony, S. T. Hwang, S. A. Rosenberg, et al. 2005. Central memory self/tumor-reactive $CD8^+$ T cells confer superior antitumor immunity compared with effector memory T cells. *Proc. Natl. Acad. Sci. USA* 102: 9571–9576.
39. Ferrari-Lacraz, S., X. X. Zheng, Y. S. Kim, Y. Li, W. Maslinski, X. C. Li, and T. B. Strom. 2001. An antagonist IL-15/Fc protein prevents costimulation blockade-resistant rejection. *J. Immunol.* 167: 3478–3485.
40. Konieczny, B. T., Z. Dai, E. T. Elwood, S. Saleem, P. Linsley, F. K. Baddoura, C. P. Larsen, T. C. Pearson, and F. G. Lakkis. 1998. IFNγ is critical for long term allograft survival induced by blocking the CD28 and CD40L T cell costimulation pathways. *J. Immunol.* 160: 2059–2064.
41. Masopust, D., V. Vezys, A. L. Marzo, and L. Lefrancois. 2001. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291: 2413–2417.
42. Sallusto, F., D. Lenig, R. Forster, M. Lipp, and A. Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401: 708–712.
43. Reinhardt, R. L., A. Khoruts, R. Merica, T. Zell, and M. K. Jenkins. 2001. Visualizing the generation of memory CD4 T cells in the whole body. *Nature* 410: 101–105.
44. Zhang, X., S. Sun, I. Hwang, D. F. Tough, and J. Sprent. 1998. Potent and selective stimulation of memory-phenotype $CD8^+$ T cells in vivo by IL-15. *Immunity* 8: 591–599.
45. Schluns, K. S., K. Williams, A. Ma, X. X. Zheng, and L. Lefrancois. 2002. Cutting edge: requirement for IL-15 in the generation of primary and memory Ag-specific CD8 T cells. *J. Immunol.* 168: 4827–4831.
46. Gorelik, L., and R. A. Flavell. 2000. Abrogation of TGFβ signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 12: 171–181.
47. Josien, R., P. Douillard, C. Guillot, M. Muschen, I. Anegon, J. Chetritt, S. Menoret, C. Vignes, J. P. Soulillou, and M. C. Cuturi. 1998. A critical role for transforming growth factor-β in donor transfusion-induced allograft tolerance. *J. Clin. Invest.* 102: 1920–1926.
48. Fu, S., A. C. Yopp, X. Mao, D. Chen, N. Zhang, D. Chen, M. Mao, Y. Ding, and J. S. Bromberg. 2004. $CD4^+$ $CD25^+$ $CD62^+$ T-regulatory cell subset has optimal suppressive and proliferative potential. *Am. J. Transplant.* 4: 65–78.
49. Ochando, J. C., A. C. Yopp, Y. Yang, A. Garin, Y. Li, P. Boros, J. Llodra, Y. Ding, S. A. Lira, N. R. Krieger, and J. S. Bromberg. 2005. Lymph node occupancy is required for the peripheral development of alloAg-specific $FoxP3^+$ regulatory T cells. *J. Immunol.* 174: 6993–7005.
50. Barthlott, T., G. Kassiotis, and B. Stockinger. 2003. T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med.* 197: 451–460.
51. Guarda, G., M. Hons, S. F. Soriano, A. Y. Huang, R. Polley, A. Martin-Fontecha, J. V. Stein, R. N. Germain, A. Lanzavecchia, and F. Sallusto. 2007. L-selectin-negative CCR7- effector and memory $CD8^+$ T cells enter reactive lymph nodes and kill dendritic cells. *Nat. Immunol.* 8: 743–752.
52. Sallusto, F., J. Geginat, and A. Lanzavecchia. 2004. Central memory and effector memory T cell subsets: function, generation and maintenance. *Annu. Rev. Immunol.* 22: 745–763.