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Suppression of Memory CD8 T Cell Generation and Function by Tryptophan Catabolism[1]

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Dendritic cell-derived indoleamine 2,3-dioxygenase (IDO) suppresses naive T cell proliferation and induces their apoptosis by catalyzing tryptophan, and hence is essential for the maintenance of peripheral tolerance. However, it is not known whether memory T cells are subject to the regulation by IDO-mediated tryptophan catabolism, as memory T cells respond more rapidly and vigorously than their naive counterparts and are resistant to conventional costimulatory blockade. In this study, we present the evidence that memory $CD8^+$ T cells are susceptible to tryptophan catabolism mediated by IDO. We found that overexpression of IDO in vivo attenuated the generation of both central memory $CD8^+$ T cells (T_{CM}) and effector memory $CD8^+$ T cells (T_{EM}) while suppressing IDO activity promoted their generation. Moreover, IDO overexpression suppressed the effector function of T_{CM} cells or T_{CM} cell-mediated allograft rejection as well as their proliferation in vivo. Interestingly, T_{CM} cells were resistant to apoptosis induced by tryptophan catabolism. However, IDO overexpression did not suppress the effector function of T_{EM} cells or T_{EM} cell-mediated allograft rejection, suggesting that T_{EM} cells, unlike T_{CM} cells, do not require tryptophan for their effector function once they are generated. This study provides insight into the mechanisms underlying the differential regulation of memory T cell responsiveness and has clinical implications for vaccination or tolerance induction. *The Journal of Immunology,* **2007, 178: 4260–4266.**

Tryptophan is an essential amino acid, the rarest of the 20 aa found in proteins (1, 2). It is an important constituent of proteins required for T cell proliferation. Indoleamine 2,3-dioxygenase (IDO),[3] an enzyme mainly expressed by APCs including macrophages, dendritic cells, and others (3, 4), catalyzes the oxidative cleavage of the indole ring of tryptophan. When infectious agents or foreign Ags invade tissues, leukocytes and lymphocytes accumulate at the site of infection and release IFN-γ that triggers IDO production by APCs (5). Tryptophan is then degraded into two major metabolites: serotonin, a neurotransmitter, and kynurenine, a series of metabolites that promote T cell apoptosis (1, 2). Therefore increased tryptophan catabolism by IDO leads to the suppression of T cell proliferation (6–8) and acceleration of T cell apoptosis (7, 9), conditions that lead to the status of peripheral tolerance induced by tolerogenic dendritic cells.

Munn et al. (10) have reported that allogeneic fetal rejection by pregnant maternal mice is prevented by IDO-mediated tryptophan catabolism as a pharmacologic inhibitor of IDO evokes a rapid T cell-mediated rejection of all allogeneic concept. They have also found that tryptophan catabolism prevents T cell-driven complement activation and inflammation during pregnancy (11), suggesting that IDO plays a key role in placenta immune privilege. IDO-expressing dendritic cells suppress allogeneic T cell proliferation in vitro by tryptophan metabolites (7) and IDO action attenuates allograft injury or rejection (12–14). Tryptophan catabolism also induces regulatory cells and is a means by which CTLA-4 signaling functions in vivo (15–17) while inhibiting IDO restores antitumor immunity (18). Hence, IDO-mediated tryptophan catabolism plays a key role in the maintenance of immunologic tolerance.

A cardinal feature of an adaptive immune response is its ability to generate long-lasting populations of memory T cells (19–21). 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 T cell costimulation blockade (22–26), function independently of secondary lymph organs (27), and are therefore an impediment to the induction of allograft tolerance (28–34). However, it is unknown whether memory T cells are susceptible to the suppression mediated by tryptophan catabolism.

In this study, we investigated whether memory $CD8^+$ T cells are subject to the regulation by IDO-mediated tryptophan catabolism. We found that overexpression of IDO in vivo attenuated the generation of both central memory $CD8^+$ T cells (T_{CM}) and effector memory $CD8^+$ T cells (T_{EM}), whereas suppressing IDO activity promoted their generation. Moreover, IDO overexpression suppressed the effector function as well as proliferation of T_{CM} cells. Interestingly, the apoptosis of T_{CM} cells was not affected by tryptophan catabolism. In contrast, IDO overexpression did not suppress the effector function of T_{EM} cells, suggesting that T_{EM} cells, unlike T_{CM} cells, do not require tryptophan for their effector function.

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[3] Abbreviations used in this paper: IDO, indoleamine 2,3-dioxygenase; T_{CM}, central memory $CD8^+$ T cells; T_{EM}, effector memory $CD8^+$ T cells; Ad-IDO, adenoviral vector coding IDO; mLN, mesenteric lymph node; CD62L, CD62 ligand; MOI, multiplicity of infection.

Materials and Methods

Mice

2C TCR-transgenic C57BL/6 mice on a Rag1 knockout ($Rag1^{-/-}$) background ($2C.Rag^{-/-}$) were generated by backcrossing 2C transgenic mice onto $Rag1^{-/-}$ mice (The Jackson Laboratory). Wild-type BALB/c and C57BL/6 mice were purchased from 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 isolation and transplantation

Islet donors were 7- to 8-wk-old BALB/c female mice. Islet recipients were 7- to 8-wk-old C57BL/6 female mice. Islets were isolated and transplanted into the subcapsular space of the right kidney of the recipient mice as previously described (31). Recipient mice were rendered diabetic by a single injection of streptozotocin (180 mg/kg; Sigma-Aldrich) 10–14 days before transplantation. Primary graft function was defined as blood glucose under 200 mg/dl for 24 h after transplantation. Graft rejection was defined as a rise in blood glucose to >300 mg/dl for 3 consecutive days.

Islet infection with adenoviral vectors

The recombinant adenoviral vector coding IDO gene (Ad-IDO, pBluescript) contains both murine IDO DNA gene and the fluorescent marker blue fluorescent protein as previously described (12). The control viral vector Ad-LacZ was purchased from Q-Biogene. The complete recombinant adenoviral vectors were propagated, amplified, and titered according to the manufacturer's manual instructions. The viruses were purified by BD Adeno-X virus purification kit (BD Biosciences), and resulted in viral stock concentrations of 2×10^9 PFU/ml. The islets were incubated in 750 μl of the serum-free medium containing adenoviral vectors at the multiplicity of infection of 100 for 90 min at room temperature. Islets were then incubated in the complete medium (RPMI 1640 supplemented with 10% FBS) at 37°C for 48 h.

RT-PCR detection of IDO gene expression in islets

Total RNA was isolated from islets infected with adenovirus using TRIzol Reagent extraction method (Invitrogen Life Technologies). The extracted RNA was subject to cDNA synthesis using the Superscript III First-Strand Synthesis system for RT-PCR (Invitrogen Life Technologies). The PCR cycling conditions were 94°C for 3 min, and 35 cycles of 94°C for 30 s, 56°C for 1 min, then 72°C for 1 min. The primers were as follows: murine IDO 5′-GCACTCAGTAAAATATCTCCTACAGAAG-3′, 5′-CTTGCTACACTAAGGCCAACTC-3′; and murine β-actin 5′-ATCCGTAAAGACCTCTATGC-3′, 5′-AACGCAGCTCAGTAACAGTC-3′. Fragments of 1.3 kb for IDO and 287 bp for β-actin were yielded by 1% agarose gel electrophoresis.

Determination of transgene expression

To determine the transgene expression, the islets infected with Ad-LacZ were detected under the brightfield microscope after standard 5-bromo-4-chloro-3-indolyl β-D-galactoside staining (β-Galactosidase Staining kit; Mirus). The blue fluorescent protein expression of islets infected with Ad-IDO was detected under fluorescent microscope by excitation with a mercury lamp and a standard 4′,6′-diamidino-2-phenylindole filter set. To further determine the specific IDO protein expression, immunohistochemistry was performed using the islets after incubation with adenoviral vector in vitro and in graft tissue sections. Briefly, the islets or graft tissues were fixed and embedded in paraffin, then cut in 4- to 6-μm sections. The sections were dried at 55°C, then deparaffinized in Xylen, followed by dehydration through graded alcohol. Endogenous peroxidase activity was blocked by 3% H_2O_2. The sections were then incubated with monoclonal mouse anti-IDO Ab (Chemicon International) at 4°C overnight. After incubation with HRP anti-mouse IgG, sections were colored using 3′-3′-diaminobenzidine (Sigma-Aldrich) and counterstained by hematoxylin.

Memory T cell preparation and phenotyping

To measure the generation of $CD8^+$ memory T cells, naive $CD8^+$ T cells ($CD8^+CD44^{low}$) from transgenic 2C $Rag^{-/-}$ mice were isolated by FACSAria cell sorter (BD Biosciences). 2C TCR-transgenic $CD8^+$ 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 $CD8^+1B2^+$. Briefly, splenocytes from 2C TCR-transgenic mice (B6) were first incubated with anti-CD8 PE and anti-CD44 FITC Abs (BD Pharmingen) and were then sorted out by FACSAria after gating on $CD8^+CD44^{low}$ population. The purity of these cells was typically >95%. These transgenic naive $CD8^+$ T cells (5×10^6) were then adoptively transferred to B6 mice that were transplanted with BALB/c islets 1 day later for the generation of memory $CD8^+$ T cells. The $CD8^+1B2^+$ 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-CD62 ligand (CD62L) allophycocyanin or anti-CD44 allophycocyanin (BD Pharmingen) as previously described (25, 35). To purify memory $CD8^+$ T cells for the adoptive transfer experiments or in vitro studies, T_{CM} ($1B2^+CD8^+CD44^{high}CD62L^{high}$) cells were isolated from spleens and lymph nodes by FACSAria cell sorting and T_{EM} ($1B2^+CD8^+CD44^{high}CD62L^{low}$) cells were isolated from livers and kidneys (25, 31).

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

Naive or memory 2C cells (2×10^4/well), isolated by FACS cell sorting from spleens and lymph nodes of recipient mice, were cultured with irradiated BALB/c spleen cells (2×10^4/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 18 h and pulsed with [^{3}H]thymidine for last 6 h. Cells were then harvested and analyzed by a scintillation counter (PerkinElmer) (36). 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 manufacturer's instructions (Invitrogen Life Technologies) (36).

Isolation of graft-infiltrating cells

Graft-infiltrating cells were isolated as described in our previous publications (25, 31). Briefly, the kidneys containing islet allografts were perfused in situ with heparinized 0.9% saline. They were then minced and digested at 37°C for 30 min in 20 ml of RPMI 1640 medium containing 5% FCS and 350 U/ml collagenase (Sigma-Aldrich). To clear the debris, cell suspensions were rapidly passed down a loosely packed glass wool column (300 mg of sterile glass wool in a 10-ml syringe), 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 washed, resuspended, and stained before FACS analysis.

Analysis of 2C cell proliferation by BrdU-labeling and apoptosis by a TUNEL method

Recipient mice were i.p. pulsed with 0.8 mg of BrdU (Sigma-Aldrich) 6 days after islet transplantation. Twenty-four hours later, mice were sacrificed and graft-infiltrating cells from the kidney were isolated and stained using anti-CD8-PE (BD Pharmingen) 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 FACSCalibur (25, 31). To detect apoptosis, graft-infiltrating cells were fixed in 2% paraformaldehyde, permeabilized with 0.1% Triton X-100 solution, and labeled with fluorescein-tagged dUTP by the TUNEL method according to the manufacturer's instructions (Roche Applied Science) (25, 31).

Treatment of mice with 1-methyl-DL-tryptophan (1-MT) and anti-CD154 (MR1) Abs

Recipient mice were treated with an IDO inhibitor, 1-MT (Sigma-Aldrich), which was implanted as a matrix-driven delivery pellet under the dorsal skin and released at 10 mg/day for 14 days (Innovative Research) (10, 11). As a control, the placebo is also implanted under the skin in the similar position. To prevent acute allograft rejection, recipient mice were i.p. treated with 0.5 mg of MR1 (BioExpress) on days 0, 2, and 4 after islet transplantation.

Statistical analysis

The analysis of allograft survival data was performed using the Mann-Whitney *U* test. Comparison of the mean was conducted using a two-tailed *t* test and ANOVA.

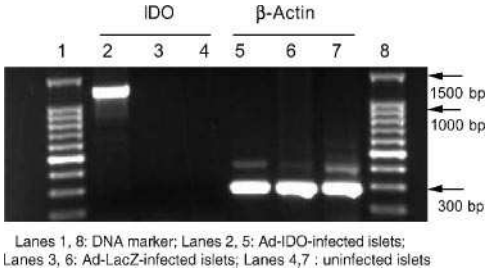

FIGURE 1. IDO gene expression in infected islets by RT-PCR. The islets were incubated in the serum-free medium containing adenoviral vectors Ad-IDO or Ad-LacZ at a multiplicity of infection of 100 for 90 min at room temperature. Islets were then washed and incubated in the complete RPMI 1640 medium supplemented with 10% FBS at 37°C for 48 h. Total RNA was isolated from the infected islets and subject to RT-PCR to measure IDO mRNA expression.

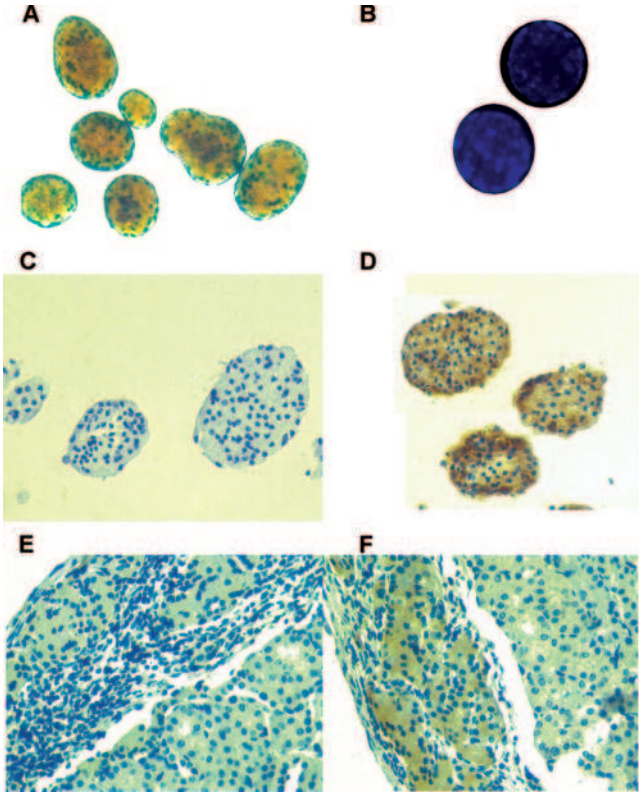

FIGURE 2. IDO protein expression in infected islets. Islets were infected with adenoviral vectors Ad-IDO or Ad-LacZ by cultures for 48 h. *A*, Islets were transfected with Ad-LacZ as detected by 5-bromo-4-chloro-3-indolyl β-D-galactoside staining (infected with Ad-LacZ at a multiplicity of infection (MOI) of 100). Islets were transfected with Ad-IDO vector containing the coding region of both IDO and blue fluorescent protein. *B*, The blue fluorescing cells were transfected with Ad-IDO (infected with Ad-IDO at an MOI of 100). Immunohistochemistry was performed using IDO-specific Ab staining in paraffin-embedded islets infected with AD-LacZ (*C*) and infected with Ad-IDO (*D*) as well as kidneys containing the infected islet allografts infected with Ad-LacZ (*E*) and infected with Ad-IDO (*F*). Ad-IDO-infected or Ad-LacZ-infected BALB/c islets were transplanted to B6 mice. Fourteen days later, the kidney was fixed, paraffin-embedded, sectioned, and then stained for IDO expression. Magnification shown at ×200.

Results

Infection of islets with Ad-IDO

To study the role for IDO expression in the generation and function of memory $CD8^+$ T cells, we first infected islet allografts with Ad-IDO. Islets infected with Ad-IDO were confirmed to express both mRNA and protein of IDO. As shown in Fig. 1, islets infected with Ad-IDO did express IDO mRNA (Fig. 1, *lane 2*) before they were transplanted, whereas those islets infected with or without Ad-LacZ did not express IDO gene (Fig. 1, *lanes 3 and 4*). Moreover, immunohistochemistry demonstrated that islets infected with Ad-IDO expressed IDO protein either before (Fig. 2*D*) or after transplantation (Fig. 2*F*), whereas islets infected with Ad-LacZ as a control did not express IDO protein either before (Fig. 2*C*) or after transplantation (Fig. 2*E*). The Ad-IDO infection of islets resulted in functionally active IDO, as measured by tryptophan depletion of culture medium and previously described (12) (data not shown).

Tryptophan catabolism severely reduces the number of memory $CD8^+$ T cells

To test whether tryptophan catabolism suppresses the generation of memory 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, thereafter referred to as $CD8^+1B2^+$. 2C naive $CD8^+$ cells were first transferred to recipient B6 mice that were transplanted with BALB/c islets 1 day later. In some groups, islet allografts were infected with AD-IDO and LacZ. In other groups, recipient mice were treated with 1-MT or placebo for the suppression of IDO activity in vivo. Ten weeks later, memory $CD8^+$ T cells ($CD8^+1B2^+CD44^{high}$) were quantified by FACS analysis. We found that the percentage of 2C memory $CD8^+1B2^+$ T cells in both spleens and mesenteric lymph nodes (mLNs) from mice that received islet allografts infected with Ad-IDO was much lower than the percentage of cells from mice that received islet allografts infected with Ad-LacZ as a control (0.4 vs 1.6% in mLN, and 0.5 vs 1.4% in the spleen) (Fig. 3*A*). In contrast, the percentage of 2C memory $CD8^+1B2^+$ T cells in both spleens and mLNs from recipient mice treated with 1-MT was higher than the percentage from recipient mice treated with placebo as a control (2.3 vs 1.6% in mLN, and 1.5 vs 1.0% in the spleen) (Fig. 3*A*). Conversely, as shown in Fig. 3*B*, the absolute number of 2C memory cells was also decreased in mice that received Ad-IDO-infected islet allografts vs mice that received Ad-LacZ-infected islet allografts (0.8 ± 0.3 vs 2.3 ± 0.6 in mLN, 3.4 ± 2.1 vs 10.1 ± 2.0 in the spleen, 1.2 ± 0.5 vs 3.4 ± 0.8 in the liver, 0.9 ± 0.3 vs 2.7 ± 0.7 in the kidney, all $p < 0.05$). However, the absolute number of 2C memory cells was increased after 1-MT treatment compared with the placebo control group (3.9 ± 0.5 vs 2.4 ± 0.4 in mLN, 13.0 ± 1.4 vs 9.3 ± 1.2 in the spleen, 5.4 ± 0.7 vs 3.6 ± 0.6 in the liver, 3.8 ± 0.5 vs 2.6 ± 0.2 in the kidney, all $p < 0.05$). It is noteworthy that very fewer 2C memory cells were detected in the bone marrow cells of transplanted mice in our allogeneic system (our unpublished observation). The $CD8^+1B2^+$2C cells generated after transplantation were confirmed to have memory phenotypes because the cells from mLNs were $CD44^{high}CD62L^{high}$, a central memory marker, whereas cells from liver organs were $CD44^{high}CD62L^{low}$, an effector memory marker (37) (Fig. 3*C*). Taken together, these data suggest that tryptophan catabolism mediated by IDO significantly suppresses the generation of both T_{EM} and T_{CM} cells.

Memory $CD8^+$ T cells, generated in the face of IDO overexpression, are functionally intact

Memory $CD8^+$ T cells generated under certain conditions are functionally impaired (38). In addition to their reduced number, memory T cells generated under IDO overexpression could be also

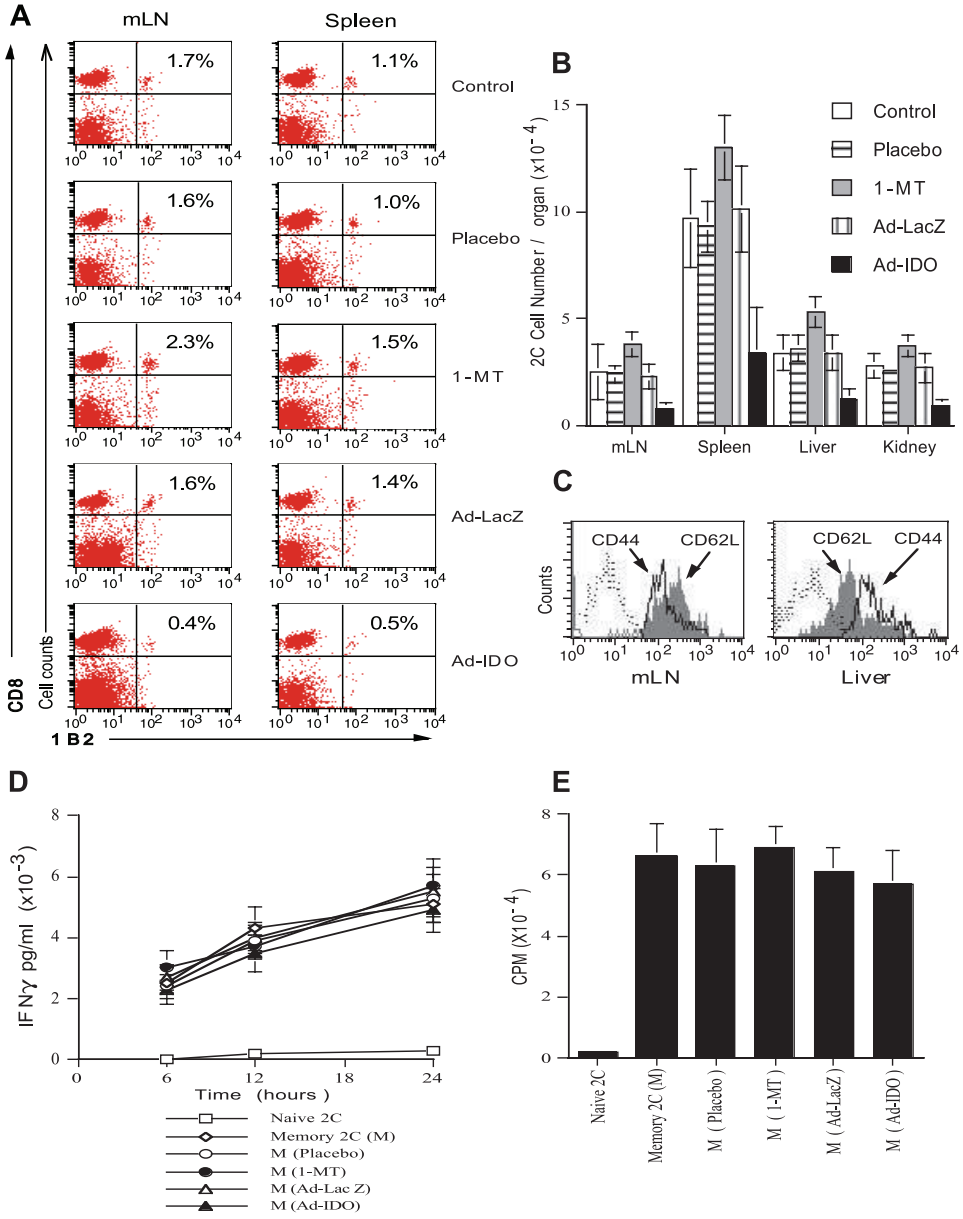

FIGURE 3. Tryptophan catabolism suppresses the generation of memory $CD8^+$ T cells. A total of 5×10^6 of naive 2C $CD8^+$ T cells ($CD8^+1B2^+CD44^{low}$) was transferred to B6 mice that were transplanted with BALB/c islets 1 day later. In some groups, islets were infected with adenoviruses Ad-IDO or Ad-LacZ before transplantation. Some recipient mice were treated with 1-MT or placebo as a control. Ten weeks later, mLNs, spleen and tissue cells were isolated and stained, and 2C memory cells ($CD8^+1B2^+CD44^{high}$) were quantified by FACS analysis. *A*, The percentage of $CD8^+1B2^+$2C memory T cells derived from mLN and spleens is shown as one representative from three independent experiments. *B*, The absolute number of 2C memory T cells per organ including both lymphoid and nonlymphoid organs. Data are shown as the mean ± SD from three independent experiments. *C*, 2C cells retrieved represent a memory phenotype. Histograms are shown for the expression of CD44 and CD62L after gating on $CD8^+1B2^+$ cells. One representative staining from three separate mice is shown. *D*, 2C ($CD8^+1B2^+CD44^{high}CD62L^{low}$) T_{EM} cells were purified by FACS cell sorting and then incubated with irradiated BALB/c splenocytes in 96-well plates for up to 24 h to measure IFN-γ production by ELISA. *E*, Similar cells were cultured for 18 h to detect 2C cell proliferation by [^{3}H]thymidine uptakes. One of three separate experiments is shown.

functionally impaired. To study whether tryptophan catabolism inhibits the generation of functional memory $CD8^+$ T cells, 2C naive $CD8^+$ cells were transferred to mice that were transplanted with BALB/c islets 1 day later as described. Ten weeks later, 2C T_{EM} cells ($1B2^+CD44^{high}CD62L^{low}$) were isolated and restimulated with irradiated BALB/c splenocytes in vitro. IFN-γ levels in the supernatant were then measured by ELISA while 2C cell proliferation was measured by [^{3}H]thymidine uptakes. As shown in Fig. 3*D*, naive 2C cells as a control did not produce IFN-γ within 24 h, whereas 2C T_{EM} cells produced a significant amount of IFN-γ even at the time point of 6 h, confirming that 2C cells generated in our system are true memory cells. Surprisingly, 2C T_{EM} cells generated under the treatment of 1-MT produced the same amount of IFN-γ as those under the placebo control (amount $\times 10^{-3}$ pg/ml at 6 h: 3 ± 0.6 vs 2.4 ± 0.6; 12 h: 3.7 ± 0.4 vs 3.9 ± 0.5; and 24 h: 5.7 ± 0.9 vs 5.3 ± 0.8; $p > 0.05$). Moreover, IDO overexpression via Ad-IDO during generation did not significantly affect the ability of the memory 2C cells to produce IFN-γ at all time points ($\times 10^{-3}$ pg/ml at 6 h: 2.3 ± 0.3 vs 2.7 ± 0.4; 12 h: 3.5 ± 0.6 vs 4.0 ± 0.5; and 24 h: 4.9 ± 0.7 vs 5.5 ± 0.8; $p > 0.05$) (Fig. 3*D*). Similarly, neither 1-MT nor Ad-IDO had significantly altered 2C T_{EM} cell proliferation (placebo: 6.3 ± 1.2 cpm vs 1-MT: 6.9 ± 0.7 cpm or Ad-LacZ: 6.0 ± 0.8 cpm vs Ad-IDO: 5.6 ± 1.1 cpm; all $p > 0.05$) (Fig. 3*E*). The production of IFN-γ by and proliferation of 2C T_{CM} cells was also not affected by IDO overexpression during generation (data not shown). These findings suggest that tryptophan catabolism impairs the generation of memory $CD8^+$ T cell number but not their function. In other words, memory $CD8^+$ T cells, although reduced in number in the face of IDO overexpression, are functionally intact once they are generated and placed back under the normal IDO environment.

Tryptophan catabolism suppresses the effector function of T_{CM} cells, but does not induce their apoptosis

To test whether tryptophan catabolism interferes with the function of memory $CD8^+$ T cells, 2C T_{CM} cells ($CD8^+1B2^+CD44^{high}CD62L^{high}$) were adoptively transferred into recipient B6 mice that received BALB/c islet allografts 1 day later. Seven days later, graft-infiltrating cells were isolated and analyzed for 2C T cell proliferation by the BrdU uptake (25, 31). As shown in Fig. 4*A*,

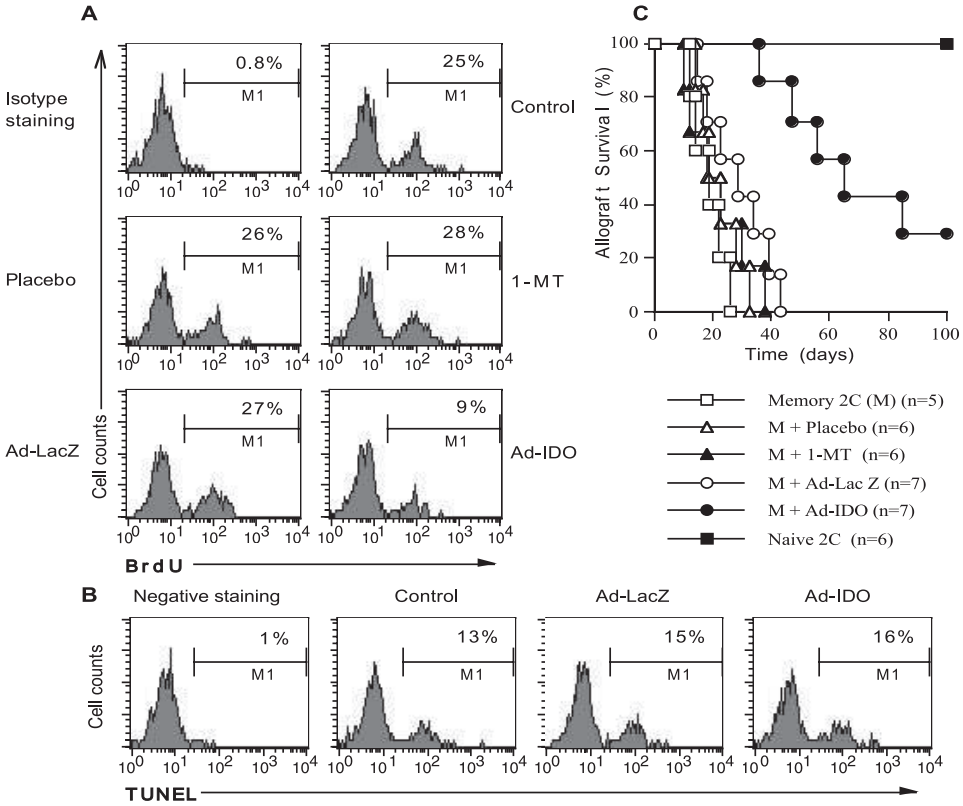

FIGURE 4. Tryptophan catabolism inhibits the effector function of T_{CM} cells but does not promote their apoptosis. A total of 1×10^6 of memory 2C T_{CM} cells ($1B2^+CD8^+CD44^{high}CD62L^{high}$), isolated from spleens and lymph nodes, was transferred into B6 mice that received BALB/c islet allografts. Some islets were infected with Ad-IDO or Ad-LacZ while some recipients were treated with 1-MT. Seven days later, graft-infiltrating cells were isolated and analyzed for 2C T cell proliferation in vivo by BrdU uptakes (*A*) and apoptosis by TUNEL (*B*). The percentage of BrdU-positive or TUNEL-positive 2C cells is shown in the histograms after gating on $CD8^+1B2^+$ cells, and one representative from three independent experiments is presented. *C*, B6 mice were transplanted with BALB/c islets and treated with 0.5 mg of MR1 (anti-CD154 Ab) on days 0, 2, and 4 to prevent acute allograft rejection. A total of 1×10^6 of 2C T_{CM} cells was then transferred to recipient B6 mice on day 5 to observe allograft rejection mediated by memory $CD8^+$ cells. Some recipient mice received 1-MT or placebo on the same day when memory 2C cells were transferred.

infection of islets with Ad-IDO severely suppressed 2C memory cell proliferation compared with infection with Ad-LacZ (BrdU-positive: 9 vs 27%). However, the suppression of IDO activity by 1-MT did not significantly alter 2C memory cell proliferation (BrdU-positive: 1-MT 28% vs placebo 26%). These data suggest that tryptophan catabolism mediated by IDO suppresses $CD8^+$ T_{CM} cell proliferation but that excessive tryptophan, resulting from 1-MT-mediated suppression of IDO, does not necessarily translate to an accelerated proliferation of the $CD8^+$ memory cells. Tryptophan metabolites promote naive/effector T cell apoptosis (7, 9). To ask whether tryptophan catabolism also induces the apoptosis of memory $CD8^+$ T cells, the similar graft-infiltrating cells as described were analyzed for their apoptosis by a TUNEL method (25, 31). As shown in Fig. 4*B*, the infection of islets with Ad-IDO did not significantly promote the apoptosis of 2C memory T cells compared with Ad-LacZ-infected group (TUNEL-positive, 16 vs 15%), indicating that tryptophan catabolism by IDO does not necessarily induce the apoptosis of memory $CD8^+$ T cells.

To further determine whether tryptophan catabolism suppresses the effector function of T_{CM} cells, we used a wild-type, but not lymphocyte-deficient, mouse model of allograft rejection mediated by 2C memory cells. B6 mice all were transplanted with BALB/c islets and treated with MR1 (anti-CD154 Ab) to prevent acute rejection. As shown in Fig. 4*C*, these recipients did not reject islet allograft within 100 days unless they received 2C T_{CM} cells because memory T cells are resistant to costimulatory blockade (22–25). The infection of islet allografts with Ad-IDO dramatically delayed allograft rejection mediated by 2C T_{CM} cells (median survival time, Ad-IDO 66 days vs Ad-LacZ 27 days; $p < 0.05$). However, The suppression of IDO by 1-MT did not significantly alter allograft survival time (median survival time, 1-MT 20 days vs placebo 21 days; $p > 0.05$). These data demonstrate that tryptophan catabolism mediated by IDO overexpression suppresses the effector function of T_{CM} cells, and that the suppression of IDO by 1-MT does not necessarily enhance their effector function.

Tryptophan catabolism does not inhibit the effector function of T_{EM} cells

To test whether tryptophan catabolism suppresses the proliferation of T_{EM} effector function, 2C T_{EM} cells were transferred into B6 mice that received BALB/c islet allografts 1 day later. Seven days later, graft-infiltrating cells were isolated and analyzed for 2C T cell proliferation by the BrdU uptake. As shown in Fig. 5*A*, IDO overexpression by islets did not suppress 2C T_{EM} cell proliferation (BrdU-positive: 21 vs 22%). To ask whether tryptophan catabolism interferes with the effector function of T_{EM} cells, B6 mice were transplanted with BALB/c islets and treated with 0.5 mg of MR1 (anti-CD154 Ab) to prevent acute rejection. Recipient mice then received 2C T_{EM} cells ($1B2^+CD8^+CD44^{high}CD62L^{low}$) to observe allograft rejection mediated by T_{EM} cells. As shown in Fig. 5*B*, overexpression of IDO by Ad-IDO-infected islets did not significantly suppress allograft rejection mediated by T_{EM} cells

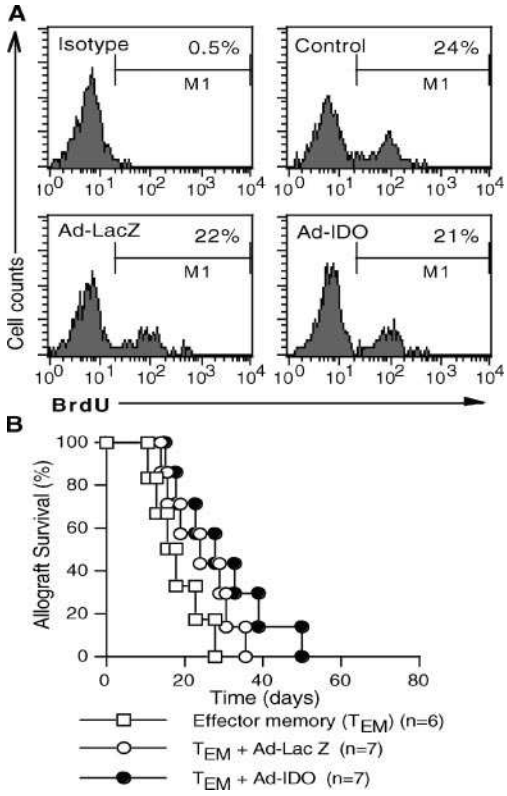

FIGURE 5. Tryptophan catabolism does not inhibit the effector function of T_{EM} cells. *A*, A total of 5×10^5 of 2C T_{EM} cells ($1B2^+CD8^+CD44^{high}$ $CD62L^{low}$) was transferred into B6 mice that received BALB/c islet allografts. Some islets were infected with Ad-IDO or Ad-LacZ. Seven days later, graft-infiltrating cells were isolated and analyzed for 2C T cell proliferation by BrdU uptakes. The percentage of BrdU-positive 2C cells is shown in the histograms after gating on $CD8^+1B2^+$ cells, and a representative experiment of three mice is presented. *B*, B6 mice were transplanted with BALB/c islets and treated with 0.5 mg of MR1 (anti-CD154 Ab) on days 0, 2, and 4 to prevent acute rejection. 2C T_{EM} cells, isolated from livers and kidney, were then transferred to recipient B6 mice on day 5 to observe allograft rejection mediated by 2C T_{EM} cells. Some islets were infected with Ad-IDO or Ad-LacZ before transplantation.

compared with Ad-LacZ control (median survival time, 24 vs 27 days; $p > 0.05$), suggesting that tryptophan catabolism does not inhibit the effector function of T_{EM} cells.

Discussion

Using a unique mouse model with transgenic donor-specific $CD8^+$ T cells, we investigated the generation and effector function of donor-specific memory $CD8^+$ T cells. We found that overexpression of IDO attenuated the generation of both T_{CM} and T_{EM} cells, whereas the suppression of IDO activity promoted their generation. Moreover, IDO overexpression suppressed the effector function as well as proliferation of T_{CM} cells. Interestingly, the apoptosis of T_{CM} cells was not affected by tryptophan catabolism, suggesting that T_{CM} cells are resistant to apoptosis mediated by tryptophan catabolism. Conversely, IDO overexpression did not suppress the effector function of T_{EM} cells. This study provided insight into the mechanisms underlying the differential regulation of memory T cell immunity. These findings are of clinical implications for the development of vaccines and design of strategies to induce transplantation tolerance. For instance, the suppression of IDO activity by 1-MT during vaccination may boost the generation of Ag-specific memory T cells. In contrast, the induction of IDO expression or direct administration of IDO enzyme may prolong allograft survival by suppressing the generation and function of allospecific memory T cells. However, IDO alone may not be sufficient to tolerize an alloantigen given that tryptophan catabolism by IDO does not inhibit the effector function of T_{EM} cells.

Our findings that IDO-mediated tryptophan catabolism controls the generation of memory $CD8^+$ T cells suggest that T cell proliferation or expansion may be essential for memory T cell generation because tryptophan deprivation suppresses T cell proliferation (6–8). It remains to be defined whether the impaired generation of memory $CD8^+$ T cells by IDO overexpression is attributed to tryptophan deprivation or apoptosis induced by tryptophan metabolites, although our data show that T_{CM} cells are resistant to apoptosis. In contrast, it is unclear why the suppression of IDO activity did not significantly enhance the effector function of $CD8^+$ T_{CM} cells while IDO overexpression severely suppressed their effector function. The fact that the suppression of IDO by 1-MT had no effect on T_{CM} cell function indicates that endogenous IDO does not play a major role in limiting the memory T cell activation under normal circumstances. It is also possible that kynurenine-derived compounds and its downstream metabolites can initiate the suppression of their effector function independently of IDO-mediated tryptophan deprivation (39).

It is believed that memory T cells are more resistant to apoptosis than their naive counterparts (40). However, it is not known whether tryptophan catabolites induce memory T cell apoptosis. Our findings suggest that T_{CM} cells are also resistant to apoptosis mediated by tryptophan catabolism. In contrast, our data demonstrate that the proliferation and effector function of T_{CM} cells are suppressed by tryptophan catabolism, indicating that IDO-mediated tryptophan catabolism suppresses T_{CM} cell function by limiting their expansion but not promoting their apoptosis. Our finding that memory $CD8^+$ cells are subject to the regulation by tryptophan catabolism supports the concept that memory $CD8^+$ T cells can undergo peripheral tolerance (41). However, our studies also show that tryptophan catabolism does not suppress T_{EM} cell function, highlighting the distinct characteristics of T_{CM} and T_{EM} cells (37, 42, 43).

We studied $CD8^+$, but not $CD4^+$, memory T cells in this allogeneic setting because 1) immune regulation in vivo results in a long-lasting $CD8^+$ memory but a declining $CD4^+$ memory pool over time (44, 45); 2) memory $CD8^+$ T cells are resistant to the conventional costimulatory blockade (22, 23, 46) and therefore could be resistant to tryptophan catabolism; and 3) memory $CD8^+$ T cells can be generated in response to an alloantigen independently of $CD4^+$ T cell help (32), suggesting that memory $CD8^+$ T cells are independent and may pose a more serious threat to tolerance induction than their $CD4^+$ counterparts. Our studies demonstrate that memory $CD8^+$ T cells are subject to the regulation mediated by tryptophan catabolism. It remains to be defined whether memory $CD4^+$ T cells are also susceptible to tryptophan catabolism.

Disclosures

The authors have no financial conflict of interest.

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