Critical Influence of Natural Regulatory CD25$^+$ T Cells on the Fate of Allografts in the Absence of Immunosuppression

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Background. Allografts are occasionally accepted in the absence of immunosuppression. Because naturally occurring CD4$^+$CD25$^+$ regulatory T cells (natural CD25$^+$ Treg cells) have been shown to inhibit allograft rejection, we investigated their influence on the outcome of allografts in nonimmunosuppressed mouse recipients.

Methods. We compared survival times of male CBA/Ca skin grafts in female CBA/Ca recipients expressing a transgenic anti-HY T-cell receptor on a RAG-1$^{+/+}$ (A1[M]RAG+) or a RAG-1$^{-/-}$ (A1[M]RAG−) background. Depletion of natural CD25$^+$ Treg cells in A1[M]RAG+ mice was achieved by in vivo administration of the PC61 monoclonal antibody. The influence of natural CD25$^+$ Treg cells on the fate of major histocompatibility complex class II-mismatched (C57BL/6× bm12)F1 skin or bm12 heart transplants in C57BL/6 recipients was also assessed. Finally, we investigated the impact of natural CD25$^+$ Treg cells on the production of T-helper (Th)1 and Th2 cytokines in mixed lymphocyte cultures between C57BL/6 CD4$^+$ CD25$^-$ T cells as responders and bm12 or (C57BL/6× bm12)F1 antigen-presenting cells as stimulators.

Results. Male allografts were spontaneously accepted by female A1(M)RAG+ mice but readily rejected by female A1(M)RAG+ mice depleted of natural CD25$^+$ Treg cells by pretreatment with the PC61 monoclonal antibody. Depletion of CD25$^+$ Treg cells also enhanced eosinophil-determined rejection of (C57BL/6× bm12)F1 skin grafts or bm12 cardiac grafts in C57BL/6 recipients. Finally, natural CD25$^+$ Treg cells inhibited the production of interleukin (IL)-2, interferon-γ, IL-5, and IL-13 in mixed lymphocyte culture in a dose-dependent manner.

Conclusion. Natural CD25$^+$ Treg cells control Th1- and Th2-type allohelper T-cell responses and thereby influence the fate of allografts in nonimmunosuppressed recipients.

Keywords: Allograft, Regulatory T cell, Tolerance.

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Although recognition of foreign antigens of organ transplants generally results in acute rejection, there are occasions when the allograft is accepted in the absence of immunosuppression. In rodent liver transplantation, such spontaneous allotolerance was linked to the emergence of regulatory cells (*1*). Indeed, there is growing evidence that the balance between effectors and regulatory T lymphocytes governs the outcome of allografts. Naturally occurring CD4$^+$CD25$^+$ regulatory T cells (CD25$^+$ Treg cells) seem to be important players in this respect (*2*, *3*). So far, the regulatory role of natural CD25$^+$ Treg cells on alloreactive responses in vivo has been well established in experimental settings of lymphopenia or immunosuppression (*3*, *4*). We analyzed the contribution of natural CD25$^+$ Treg cells to the acceptance of allografts in nonimmunosuppressed mouse recipients of skin or heart allografts. Our observation that depletion of CD25$^+$ Treg cells enabled rejection of HY-mismatched or major histocompatibility complex (MHC) class II-mismatched allografts in otherwise refractory donor-recipient combinations led us to determine the influence of natural CD25$^+$ Treg cells on T-helper (Th)1-type and Th2-type allohelper T-cell responses.

MATERIALS AND METHODS

Mice

Female wild-type C57BL/6 mice, aged 4 to 8 weeks, were obtained from Harlan (Horst, The Netherlands). C57BL/6 CH-2bm12 (bm12) mice were purchased from Jackson Laboratories (Bar Harbor, ME). (C57BL/6×

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bm12)F1 mice were obtained in our animal facility by the crossbreeding of bm12 and C57BL/6 parents. A1(M) T-cell receptor (TCR)-transgenic mice and A1(M).RAG-1$^{-/-}$ TCR-transgenic mice on CBA/Ca ($H2^k$) background (*5*) were bred and maintained in specific pathogen-free conditions at the Sir William Dunn School of Pathology (Oxford, UK). All procedures were conducted in accordance with the Home Office Animals (Scientific Procedures) Act of 1986.

Skin Grafting

Mice were anesthetized with a mixture of xylazine (Rompun) 5% and ketamine (Imalgene) 10% in phosphate-buffered saline. A total of 120 μL per 20 g of body weight was injected intraperitoneally. Skin grafting was performed as previously described (*6*). In short, skin grafting was conducted by grafting full-thickness tail skin (1×1 cm) on the lateral flank. Grafts were observed on alternate days after the removal of the bandage on day 7 and considered rejected when more than 75% of epithelial breakdown had occurred.

Cardiac Allografts

Cardiac allografts were heterotopically transplanted into the recipient's abdomen as previously described (*7*). Donor-to-recipient aorta and donor pulmonary artery-to-recipient inferior vena cava were anastomosed end-to-side with a 10-0 continuous nylon suture. Transplant function was assessed daily by abdominal palpation. Rejection was defined as the complete absence of a palpable beat. At rejection, the grafts were taken for histology.

Histologic Studies

Histology was performed on tissue sections stained with hematoxylin-eosin (H&E) after fixation in 10% neutral formalin solution and paraffin embedding.

Production of Cytokines in Mixed Leukocyte Cultures

$CD4^+$ T cells purified from spleen and lymph node were used as responders (1×10^6/mL) and seeded with 6×10^5/mL tumor necrosis factor (TNF)-α stimulated dendritic cells (DCs) (stimulators) in 48-well flat-bottom plates (Nunc, 150687, Roskilde, Denmark). $CD4^+$ T cells were positively separated by magnetic cell sorting with CD4 (L3T4) microbeads according to the protocol provided by the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany). When indicated, stimulator cells consisted of T-cell–depleted and irradiated (2,000 Rad) splenocytes, whereas responder cells included a constant number of $CD4^+CD25^-$ T cells and increasing numbers of $CD4^+CD25^+$ T cells. The purification of $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells were separated by magnetic cell sorting with the $CD4^+CD25^+$ Regulatory Cell Isolation Kit (Miltenyi Biotec). Briefly, $CD4^+$ T cells were purified by negative selection, and $CD4^+CD25^+$ T cells were separated by positive selection with phycoerythrin (PE)-conjugated anti-CD25 monoclonal antibody (mAb) and anti-PE magnetic beads following the manufacturer's instructions. We used the negative fraction as $CD4^+CD25^-$ cells. Cultures were incubated at 37°C in 5% CO_2 atmosphere. Culture medium consisted of Roswell Park Memorial Institute (RPMI) 1640 supplemented with 20 mM HEPES, 2 mM glutamine, 1 mM nonessential amino acids, 5% heat-inactivated fetal calf serum, sodium pyruvate, and 2-mercaptoethanol. Supernatants were harvested after 24, 48, and 72 hr of culture to determine interleukin (IL)-2, interferon (IFN)-γ, IL-5, and IL-13 levels. Cytokine quantification was performed with a commercially available enzyme-linked immunosorbent assay kit (Duoset, R&D systems, Minneapolis, MN) for IL-2, IFN-γ, and IL-13, and with the Opt EIA set (PharMingen, San Diego, CA) for IL5. The detection limits were 8 pg/mL for IL-2, 15 pg/mL for IL-5, 30 pg/mL for IFN-γ, and 40 pg/mL for IL-13. When indicated, T-cell–depleted irradiated spleen cells were used as stimulators. Before stimulator irradiation (2,000 rad), spleen cells were depleted of $CD4^+$ and $CD8^+$ T cells through the incubation with anti-CD4 mAb (GK1.5 clone) and anti-CD8 mAb (H35 clone), followed by addition of baby rabbit complement.

Generation of Bone Marrow-Derived Dendritic Cells

To generate DCs from bone marrow cultures, we used a modified protocol described by Buonocore et al. (*7*). Briefly, bone marrow was flushed from the femurs and tibiae of mice, disintegrated by vigorous pipetting, filtered through a nylon mesh, and depleted of red blood cells with ammonium chloride. At day 0, bone marrow progenitors were seeded in a six-well plate at the rate of 1×10^6 per well in 4 mL of RPMI 1640 (BioWhittaker) medium containing 10% heat-inactivated fetal bovine serum (SB0012; BioWhittaker), 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 2 mM glutamine, 1 mM nonessential amino acids (BioWhittaker), sodium pyruvate (BioWhittaker), 2-mercaptoethanol, and 20 ng/mL of recombinant murine granulocyte macrophage-colony stimulating factor (rmGM-CSF). On day 3, another 4 mL of complete medium containing 20 ng/mL rmGM-CSF was added to each well. On days 6 and 8, half of the culture supernatant was collected and centrifuged, and the cell pellet was resuspended in 4 mL fresh medium supplemented with 20 ng/mL rmGM-CSF and returned to the original well. On day 10, DCs were harvested by gentle pipetting. On days 3, 6, and 8, 10 ng/mL of TNFα (R&D systems Europe, UK) was added. Cells were analyzed on a FACScalibur flow cytometer (Becton Dickinson) and revealed a phenotype compatible with TNF-α stimulation.

Antibody Preparation

Anti-CD25 (PC61; rat immunoglobulin [Ig]G1 mAb) and rat-antimouse control mAb (LO-DNP-2; anti-DNP rat IgG1 mAb, provided by Dr. H. Bazin, Experimental Immunology Unit, Université Catholique de Louvain, Louvain, Belgium) were produced as ascites in nude mice (*6*). mAb concentrations were determined by a specific enzyme-linked immunosorbent assay (LO-IMEX, Université Catholique de Louvain). PC61 mAb selectively binds $CD25^+$ T cells and has been used successfully in numerous studies to deplete $CD25^+$ T cells (*8*).

In Vivo Treatments

CD25 depletion was achieved through thymectomy and anti-CD25 mAb (PC61) injections (*8*). Thymectomy was conducted as described by Monaco et al. (*9*). Briefly, a longitudinal incision was made on the anterior surface of the neck, and the thymus was removed as two intact lobes by the application of negative pressure through a tip inserted in the ante-

rior mediastinum. Next, three injections every other day of anti-CD25 1 mg (PC61) were made 1 week before transplantation. Thymectomized control mice received the control rat IgG mAb according to the same schedule. CD25 T-cell depletion was measured by FACScalibur flow-cytometry (Becton Dickinson) before and after skin transplantation. For phenotypic analysis, spleen cells and lymph node cells were incubated with the anti-CD25 fluorescein isothiocyanate-conjugated mAb (7D4, Pharmingen) and the anti-CD4 PE-labeled (GK1.5, Pharmingen). Anti-CD4 (GK1.5) and anti-CD8 (H35) mAb were used as culture cell line supernatants.

Statistical Analysis

Graft survival curves and cytokine levels were compared by the log-rank test and Mann-Whitney nonparametric test, respectively. All comparisons were two-tailed.

RESULTS

Role for Natural CD25$^+$ Treg in Spontaneous Acceptance of Male Skin Grafts in Anti-HY T-Cell Receptor Transgenic Female Recipients

As previously shown (*5*), 100% of female CBA/ca A1(M).RAG-1$^{-/-}$ mice, which express an anti-HY transgenic TCR, rejected congenic male skin graft 15 days after transplantation (Fig. 1A). To the contrary, *A1(M)RAG+* females did not reject male skin grafts (Fig. 1A). Given the known absence of foxP3$^+$, CD25$^+$ T-cells in the A1(M).RAG-1$^{-/-}$ (*5*), we examined the effect of CD25$^+$ T-cell depletion on male skin graft survival in *A1(M)RAG+ mice*. Pretransplant injection of PC61 mAb resulted in skin graft rejection in the majority of the *A1(M)RAG+* recipients (Fig. 1A). In parallel, we verified that CD4$^+$CD25$^+$ T-cell depletion elicited by PC61 mAb was successful (Fig. 1B). These results provide evidence that tolerance of male skin grafts in female transgenic *A1(M)RAG+* mice critically depends on natural CD25$^+$ T-reg cells.

CD25$^+$ T-Cell Depletion Prevents Acceptance of (C57BL/6× bm12)F1 Skin Grafts in C57BL/6 Recipients

Given the impact of natural CD25$^+$ T cells on graft rejection in this somewhat "contrived" TCR transgenic model, we investigated a more conventional donor–recipient combination in which bm12 donors and C57BL/6 recipients differ in three amino acids in the unique MHC class II molecule (I-A) they express. This disparity is sufficient to elicit acute rejection of bm12 skins by C57BL/6 recipients (*6*). (C57BL/6× bm12)F1 skin grafts that express both donor and recipient I-A were spontaneously accepted in approximately 50% of C57BL/6 recipients (Fig. 2A). Mice were depleted of natural CD25$^+$ Treg cells by thymectomy followed by three injections of PC61 mAb, a regimen that resulted in almost complete disappearance of CD25high T cells in spleen and lymph nodes (data not shown). As shown in Figure 2A, 17 of 18 CD25$^+$ T-cell–depleted animals acutely rejected their allografts within 20 days, whereas thymectomized mice injected with a control mAb behaved no differently than intact mice. At the histologic level, skin grafts rejected by CD25$^+$ T-cell–depleted recipients displayed necrotic changes with significant infiltrates of eosinophil-enriched leukocytes (Fig. 2B and C). In parallel, we found that natural CD25$^+$ Treg cell depletion did not modify the rapid rejection of single MHC class II-disparate bm12 skin allografts (Fig. 2A).

CD25$^+$ T Cells Control Rejection of bm12 Heart Allografts in C57BL/6 Recipients

In contrast with skin transplants, heart allografts in the C57BL/6-bm12 strain combination undergo low-grade and delayed rejection (*7*, *10*). We wondered whether this slow kinetic of rejection reflects a participation of natural CD25$^+$ Treg. To address this question, bm12 heart allografts were transplanted into C57BL/6 recipients depleted

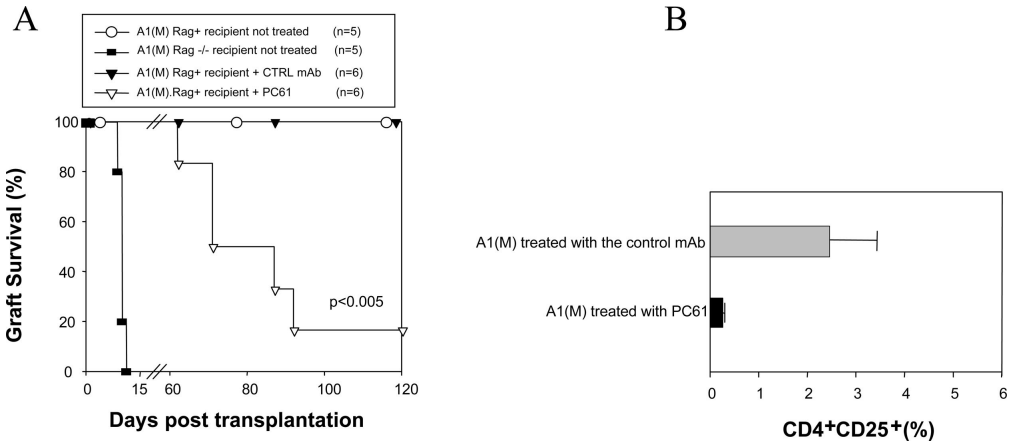

FIGURE 1. CD25-depletion of female *A1(M)RAG*$^+$ recipient mice triggers rejection of male CBA/Ca skin grafts. (A) A1(M).RAG$^{-/-}$ mice that do not contain CD25$^+$ natural Treg cells (■, n=5) were grafted with male CBA/Ca RAG1$^{-/-}$ skin. Male skin graft survival in untreated A1(M).RAG+ is shown (○, n=5). A1(M).RAG+ (△, n=6) mice were depleted of CD25$^+$ T cells through repeated injections of 1 mg of anti-CD25 monoclonal antibody (mAb) at days 17, 15, and 13 before transplantation. Control A1(M).RAG+ (▲, n=6) mice were injected with 1 mg of isotype-matched indifferent (CTRL) mAb according to the same schedule. All mice were grafted with male CBA/Ca RAG1$^{-/-}$ skin at day 0. (B) The proportion of peripheral CD4$^+$ T cells from A1(M).RAG$^{+/+}$ mice treated with anti-CD25 mAb or isotype control that expressed CD25 on their surface at the time of transplantation (at least three mice/group, plotted as mean ± standard deviation).

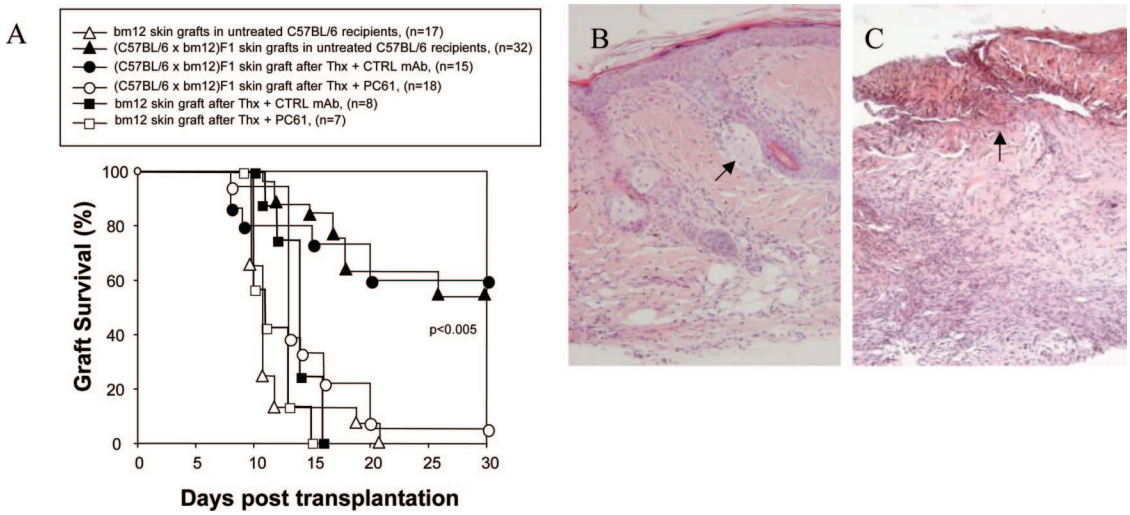

FIGURE 2. $CD25^+$ T-cell depletion affects (C57BL/6× bm12)F1 skin allograft survival but not single major histocompatibility complex (MHC) class II disparate bm12 skin grafts. (A) bm12 and (C57BL/6× bm12)F1 skin graft survival in uninjected nonthymectomized animals (△ n=17 and ▲ n=32, respectively). Five-week-old C57BL/6 recipient mice were thymectomized (Thx) and depleted of $CD25^+$ T cells with three consecutive injections of 1 mg PC61 mAb at days 11, 9, and 7 before transplantation. Control animals were thymectomized and injected with isotype-matched indifferent (CTRL) mAb. At day 0, CD25-depleted animals were grafted with a (C57BL/6× bm12)F1 skin (○, n=18) or a bm12 skin (□, n=7). At the same time, control mice were grafted with either a (C57BL/6× bm12)F1 skin (●, n=15) or a bm12 skin (■, n=8). (B) Histologic aspect of accepted (C57BL/6× bm12)F1 skin allografts 30 days after transplantation in control animals. The derma and epidermis are healthy. The derma contains intact hair follicles with preserved sebaceous glands (*arrow*) and few infiltrating leukocytes. This aspect is representative of all accepted (C57BL/6× bm12)F1 skin grafts (hematoxylin-eosin [H&E] staining, magnification ×100). (C) Histology of rejected (C57BL/6× bm12)F1 skin allografts after $CD25^+$ T-cell depletion. The epidermal layer is broken down (*arrow*) and hair follicles disappeared in a dense leukocyte infiltrate (H&E staining, magnification ×100).

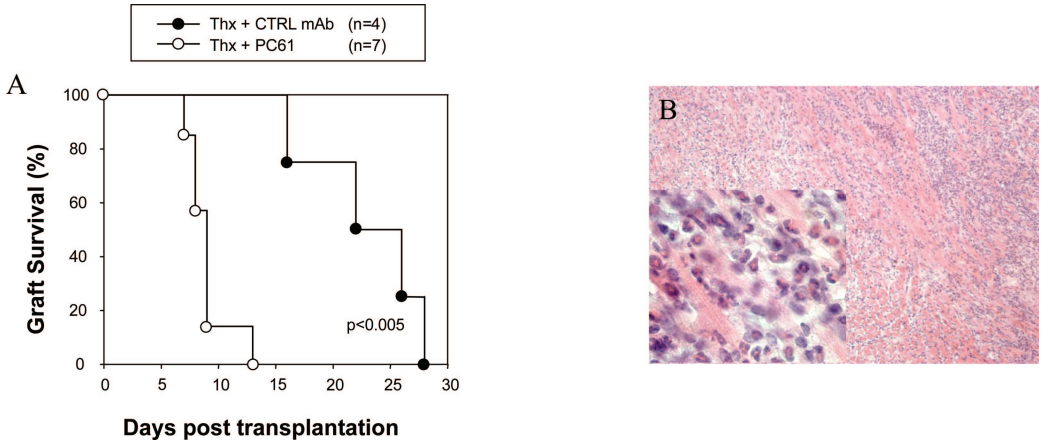

FIGURE 3. $CD25^+$ T-cell depletion of C57BL/6 recipients induces acute bm12 heart allograft rejection. (A) Five-week-old C57BL/6 recipients were thymectomized (Thx) and depleted of $CD25^+$ T cells as described in Figure 2. At day 0, $CD25^+$ T-cell–depleted animals (○, n=7) or control mice, thymectomized and injected with isotype-matched indifferent (CTRL) mAb (●, n=4), were transplanted with a bm12 heart allograft. (B) Representative histology of rejected heart allografts at day 7 posttransplantation in $CD25^+$ T-cell–depleted C57BL/6 recipients (H&E staining, magnification ×100 and ×1,000 for the inset). Dense leukocyte infiltrate invades myocardium and dislocates muscle fibers causing hemorrhages. Massive eosinophil infiltrate is recognized by their typical red (eosinophilic) intracytoplasmic granules (*left inset*).

or not of natural $CD25^+$ Treg cells. The median survival time in thymectomized mice injected with a control mAb was 27.5 (16–28) days (Fig. 3A). Mice injected with the PC61 mAb rejected more rapidly with a median graft survival time of 8 (7–13) days ($P<0.05$), also implicating a regulatory action of natural $CD25^+$ Treg in this combination (Fig. 3A). At the histologic level, acutely rejected cardiac allografts in natural $CD25^+$ Treg-cell–depleted mice displayed large necrotic areas and infiltration by eosinophils and mononuclear cells (Fig. 3B).

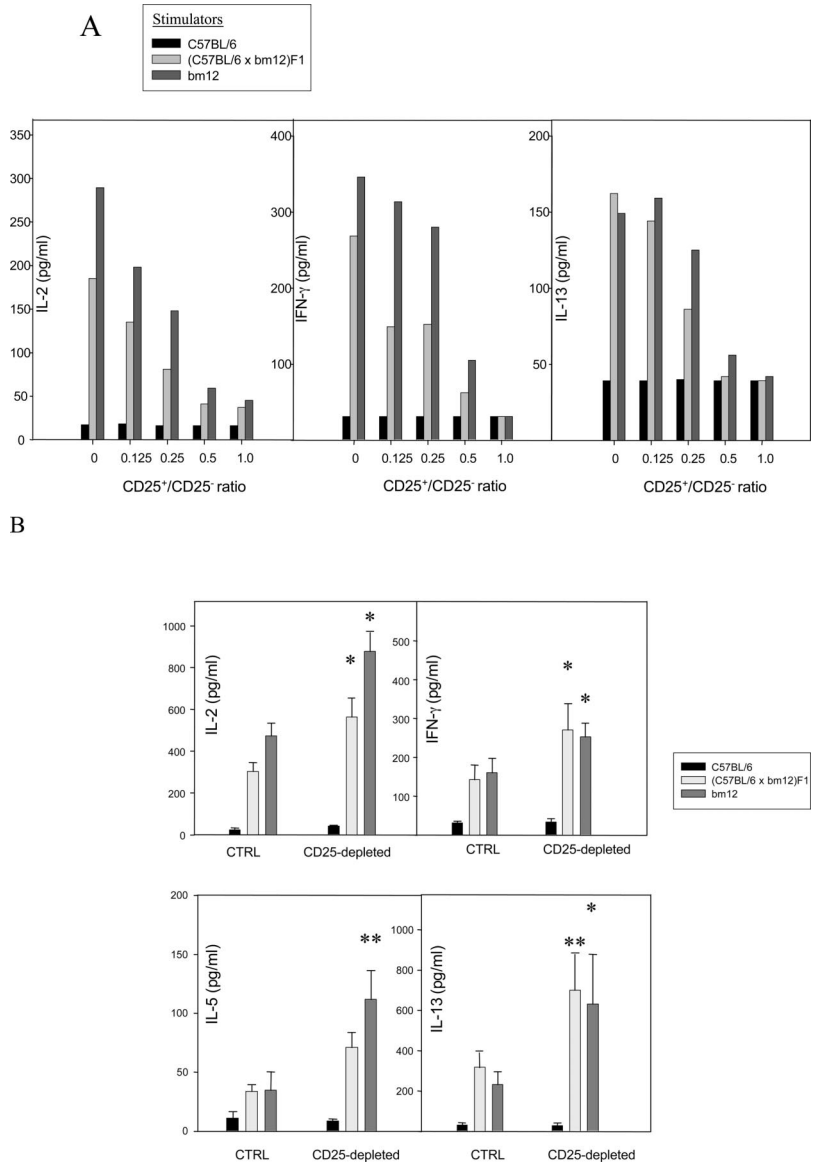

FIGURE 4. Natural CD25$^+$ Treg cells control the production of both T-helper (Th)1 and Th2 cytokines in primary mixed lymphocyte culture (MLC). (A) Graded numbers of C57BL/6 natural CD25$^+$ Treg cells were added to MLC between purified C57BL/6 CD4$^+$CD25$^-$ T cells as responders and syngeneic C57BL/6 (*black*), bm12 (*dark grey*), or (bm12× C57BL/6)F1 (*light grey*) T-cell–depleted irradiated spleen cells as stimulators. All of the T cells (CD25$^+$ or CD25$^-$) came from naïve animals. Interleukin (IL)-2 production at 48 hr and interferon (IFN)-γ and IL-13 productions at 72 hr were measured. Cytokine productions by natural CD25$^+$ Treg cells in the presence of syngeneic, bm12, or (bm12× C57BL/6)F1 stimulators were undetectable (not shown). (B) Primary MLC of CD4$^+$ T cells obtained from control mice (CTRL) injected with isotype-matched indifferent mAb (n=16) or natural Treg-cell–depleted mice (referred as CD25-depleted, n=12). In each condition, 2×10^6 purified CD4$^+$ T cells were used as responders and 6×10^5 tumor necrosis factor (TNF)-α stimulated bone marrow-derived dendritic cells (DCs) as stimulators according to "Material and Methods": C57BL/6 (*black*), bm12 (*dark grey*), (bm12xC57BL/6)F1 (*light grey*). IL-2 production was quantified at 48 hr; IFN-γ, IL-13, and IL-5 productions were measured at 72 hr. Results were expressed as mean ± standard error of mean (*$P<0.05$; **$P<0.01$).

Natural CD25$^+$ Treg Cells Control the Production of Both Th1 and Th2 Cytokines in Primary Mixed Lymphocyte Culture

The histologic pictures of MHC class II-disparate grafts rejected by mice depleted of natural Treg cells suggested the involvement of Th2-type responses, because graft eosinophilic infiltrates were previously shown to depend on IL-5 and IL-4 (*11*). This led us to investigate the impact of CD25$^+$ Treg cells on the production of Th1- and Th2-type cytokines in mixed lymphocyte culture (MLC). For this purpose, we first added graded numbers of C57BL/6 natural CD25$^+$ Treg cells to MLC between purified C57BL/6 CD4$^+$CD25$^-$ T cells as responders and bm12 or (bm12× C57BL/6)F1 stimulators. As shown in Figure 4A, natural CD25$^+$ Treg cells inhibited in a dose-dependent manner the production of IL-2, IFN-γ, and IL-13 in response to both types of stimulators. We next compared the responses in primary MLC of CD4$^+$ T cells obtained from intact mice or natural Treg-cell–depleted mice (Fig. 4B). In vivo depletion of natural CD25$^+$ Treg cells in C57BL/6 mice enhanced the capacity of their CD4$^+$ T cells to secrete IL-2, IFN-γ, IL-13, and IL-5 in response to bm12 or (bm12× C57BL/6)F1 DCs, providing further evidence that natural CD25$^+$ Treg cells control the development of both Th1 and Th2 alloreactive responses.

DISCUSSION

Natural CD25$^+$ Treg cells are known to control immune responses against self- and non-self antigens (*2*, *3*, *12*). Indeed, depletion of natural CD25$^+$Treg cells was shown not only to promote autoimmunity but also to modify the course of infectious diseases, enhance antitumor responses, and prevent successful pregnancy by interfering with maternal tolerance to fetal alloantigens (*13–19*). The latter finding is in keeping with the present report in which we demonstrate that the fate of allografts can be critically influenced by the presence of natural CD25$^+$ Treg cells.

The impact of natural CD25$^+$ Treg cells on the rejection rate of (C57BL/6× bm12)F1 skin grafts, but not on the

kinetics of rejection of bm12 skin grafts, might be related to a reduced antigenic load in the (C57BL/6× bm12)F1 combination. Because the repertoire of natural $CD25^+$ Treg TCR cells is biased toward self-reactivity (*20*), one could also speculate that their activation requires the expression of self-MHC on the antigen-presenting cell. Although there is evidence that the anti-bm12 response of C57BL/6 mice merely depends on the direct pathway of allorecognition (*21*), we observed that natural $CD25^+$ Treg cells also control rejection of single MHC class II disparate bm12 heart allografts, in which the lower grade of rejection compared with skin allografts may be related to reduced numbers of resident DCs and lower expression of MHC class II molecules in heart tissue compared with skin (*22–24*). The capacity of natural $CD25^+$ Treg to regulate responses to single MHC class II disparate bm12 cells was clearly established in our series of MLC experiments in which Treg cells were added to or depleted from C57BL/6 $CD4^+$ T cells. Because natural $CD25^+$ Treg cells require activation signals to exert their suppressive function, we assume that they were activated by $I\text{-}A^{bm12}$ because of cross-reactivity with $I\text{-}A^b$.

Because both Th1-type and Th2-type allohelper T-cell responses were previously found to contribute to acute rejection of bm12 allografts in C57BL/6 recipients (*6*, *7*, *24*), our findings suggested that both types of responses were suppressed by natural $CD25^+$ Treg cells. This was confirmed by: (1) the enhanced production of IL-2, IFN-γ, IL-5, and IL-13 by $CD4^+$ T cells from Treg-cell–depleted C57BL/6 mice on in vitro stimulation by bm12 alloantigen, and (2) the dose-dependent suppression of both IFN-γ and IL-13 production following addition of purified natural $CD25^+$ Treg cells to MLC between C57BL/6 $CD4^+CD25^-$ T cells and bm12 or (bm12× C57BL/6)F1 antigen-presenting cells. These observations are in keeping with previous reports indicating that natural $CD25^+$ Treg cells suppress Th2 and Th1 responses during experimental *Leishmania major* infection or colitis in mice (*18*). On the other hand, depletion of natural $CD25^+$ Treg cells was found to enhance Th1 activities in other settings (*25*, *26*). Differences in the degree of DC maturation and in the timing of natural $CD25^+$ Treg-cell depletion might explain these conflicting results. Indeed, Stassen et al. (*27*) recently specified parameters governing the regulatory capacity of natural $CD25^+$ Treg cells on development, functional activation, and proliferation of Th2 cells.

Although obtained in specific experimental models, our findings might be relevant to immunosuppression strategies in clinical transplantation. There is recent evidence that $CD25^+$ Treg cells contribute to donor-specific hyporesponsiveness in stable renal transplant recipients (*28*). Furthermore, the frequency of $CD4^+CD25^+$ T cells in lung transplant recipients was found to correlate with graft outcome (*29*). Therapies harnessing the suppressive activity of natural $CD25^+$ Treg cells might therefore promote long-term acceptance of solid-organ transplants, whereas agents interfering with their survival might be detrimental. In this context, the long-term consequences of anti-CD25 mAb-based induction therapy should be revisited because there is evidence that these agents might exert some depleting effect (*30*). As suggested by our data and a previous report showing that anti-CD25 mAb treatment triggered or delayed graft rejection in mice according to the nature of donor-recipient incompatibility (*31*), the beneficial effect of IL-2 receptor blockade achieved by anti-CD25 mAbs might indeed be counterbalanced by a negative influence on natural Treg-cell activities.

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