A glucocorticoid amplifies IL-2-induced selective expansion of CD4⁺CD25⁺FOXP3⁺ regulatory T cells in vivo and suppresses graft-versus-host disease after allogeneic lymphocyte transplantation

Yanhui Xie¹*, Min Wu¹, Runhua Song¹, Jiexian Ma¹, Yi Shi², Wenming Qin², and Youxin Jin²*

¹Department of Hematology, Huashan Hospital Affiliated to Fudan University, Shanghai 200040, China
²Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai 200031, China

*Correspondence address. Tel: +86-21-52889999; Fax: +86-2162489191; E-mail: xyh@medmail.com.cn (Y.X.); Tel: +86-21-54921222; Fax: +86-2154921011; E-mail: yxjin@sibs.ac.cn (Y.J.)

Keywords glucocorticoid; dexamethasone; interleukin-2; regulatory T cell; graft-versus-host disease

Received: May 8, 2009 Accepted: June 1, 2009

Introduction

Allogeneic stem cell transplantation (SCT) remains the definitive immunotherapy for malignancy, while morbidity and mortality due to graft-versus-host disease (GVHD) remain the major barriers to its advancement [1]. The early complications of allogeneic SCT are now viewed as an allogeneic immune response primarily mediated by donor T lymphocytes with specificity against alloantigens expressed by host antigen-presenting cells in the presence of proinflammatory cytokines [2]. Up to 30% of the recipients of stem cells or bone marrow transplants from HLA-identical related donors as well as most patients receiving cells from other sources (matched, unrelated, non-HLA-identical siblings, cord blood) will develop >Grade 2 acute GVHD (aGVHD) despite immunosuppressive prophylaxis [1,3]. Steroids, in combination with cyclosporine or, to some extent, tacrolimus, have been the standard therapy for the initial management of aGVHD. The mechanism is unclear, but is most likely related to the suppression of cytokines and lymphocytic activity [1]. About one-third of patients with aGVHD respond to initial therapy, leaving 10–50% of all patients who underwent transplants in need of secondary treatment [4]. Steroid-refractory aGVHD has a poor prognosis of <10% survival for 1 year [5].

Polyclonal [6,7] and monoclonal [8–11] antibodies are the most widely used secondary agents and are aimed at.
inactivating alloreactive donor T lymphocytes, NK cells, host antigen-presenting cells, cytokines, and/or cytokine receptors. They may also be used for tissue repair in the recipient. Many controlled clinical trials found these second-line therapeutic strategies to be useful, but there is no significant benefit in terms of long-term survival, and there is apparent increase in infectious mortality [6–11] when using this course of treatment.

Several murine studies indicated the possibility of preventing GVHD by administering naturally occurring regulatory T (Treg) cells [12–15].

Treg cells are a minor subpopulation of T cells that represent 5–10% of the normal T cell complement in mice and human [13,16,17]. Treg cells develop in the thymus and migrate to the periphery, where their main role is to suppress self-reactive T cells that escape negative selection in the thymus [18]. To maintain a fine balance between peripheral tolerance to autoantigens and the ability to initiate protective immune responses against infectious agents, a network of CD4+ Treg cells exists that plays an important role in various contexts and inflammatory settings [19]. Naturally occurring regulatory T cells constitutively express the α-chain of the high-affinity interleukin (IL)-2 receptor, CD25 [20], and FOXP3, and preferentially express other surface markers including CD103, CD62L, GITR, CTLA-4, neuropilin-1, and lymphocyte activation gene-3 [19]. Mutations in the FOXP3 gene cause a fatal autoimmune and inflammatory disorder in scurfy mice, and immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome in humans [21]. Subtle alterations in Treg functions have also been reported in patients with multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, autoimmune polyglandular syndromes, and allergic reactions [22].

In mouse models of allogeneic SCT, depletion of Tregs within grafts accelerates GVHD [13,14,23], whereas adoptive transfer of large numbers of donor-derived Treg cells effectively prevents GVHD [13,14]. The composition of Treg/Teff was rather important in the incidence of aGVHD [14]. The finding that graft Treg content may predict the risk of GVHD was also reported in human following SCT assessments [24–26].

Although suppression of the proliferation and function of conventional T cells might be predicted to impair cellular graft-versus-leukemia (GVL) effects, a number of studies have demonstrated the persistence of immune-mediated GVL effects [1]. Nguyen [15] demonstrated that Treg infusion 2 days ahead of infusion with conventional effector T cells and Treg subsets at almost physiological doses (10:1) prevented dramatic early proliferation of effector T cells, but allowed the persistence of sufficient numbers to mount an effective GVL response. However, the clinical application of Treg cells is limited by the difficulty of obtaining sufficient numbers of CD4+CD25+FOXP3+ cells [27].

Several approaches to deplete [2,28] or anergize alloreactive T cells [13,26] have been developed. Methods to generate or expand Treg cells in vitro have been reported, such as the direct expansion of purified CD4+CD25+ Treg cells [29,30] and conversion from CD4+CD25− T cells [31–33]. Almost all grafts engineered for GVHD prophylaxis are generated ex vivo by the isolation and manipulation of graft subpopulations. These approaches may cause pollution, damage, and deactivation of the donor cells, thus increasing the possibility of infection. We are currently searching for a way to expand donor Treg cells in vitro to provide a novel approach for the treatment of GVHD.

Glucocorticoid (GC) treatment (dexamethasone, or Dex) has been reported by Chen et al. [34] to amplify IL-2-mediated selective in vivo expansion of Treg cells and thereby inhibit the development of experimental autoimmune encephalomyelitis. GCs are potent anti-inflammatory agents that block cytokine production. Inhibition of Jak-STAT signaling by IL-2 and related cytokines might be a mechanism of GC action [35], suggesting that inhibition of cytokine signaling contributes to the clinical efficacy of these agents. CD4+CD25+ T cells express higher levels of glucocorticoid receptors (GRs) and Bcl-2, and are more resistant to Dex-mediated cell death than are CD4+CD25− T cells [36].

IL-2 was identified based on its potent T cell growth factor activity and is widely considered to be a key cytokine in T cell-dependent immune responses. IL-2 is produced by activated T lymphocytes and exerts its biological effect by binding the high-affinity IL-2 receptor (IL-2R). IL-2 was found to be essential for the generation, clonal expansion, survival, and function of Treg cells [18].

Furthermore, IL-2 selectively protects CD4+CD25+ T cells from Dex-induced cell death, while IL-7 and IL-15 do not exert preferential protective effects [36]. Therefore, signaling through IL-2R by IL-2 may induce the expansion of both Treg cells and effector T (Teff) cells, with Treg cells being more resistant to Dex-induced cell death in an IL-2 environment. A combination of these two agents could selectively expand Treg cells in vivo. This hypothesis was tested by
assessing the effects of Dex and IL-2 on Treg cells in a murine model for GVHD after MHC-unmatched allogeneic lymphocyte transplantation. We demonstrated that infusion of donor cells pre-treated with Dex and IL-2 inhibited GVHD after allogeneic transplantation.

Materials and Methods

Animals
Male C57BL/6 (H-2Kb) and female BALB/c (H-2Kb) mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China). All mice were 8–12 weeks old. The care of all experimental animals was in accordance with institutional guidelines (National Standard of PRC GB14925-2001). Mice were maintained on specific pathogen-free standard laboratory food and water.

Antibodies and flow cytometry
The following reagents were used for flow cytometric analysis: Mouse Regulatory T cell Staining Kit #3 (w/PE-Cy5 FOXP3 FJK-16s, FITC CD4, PE CD25; Treg Kit) (eBioscience, San Diego, USA), PE anti-mouse CD152 (CTLA-4, CTLA4; eBioscience), FITC anti-mouse GITR (TNFRSF18; eBioscience), Mouse Anti-Mouse H-2Kb MHC Class I (private; Caltag), PE Armenian Hamster IgG Isotype Control (eBioscience), Mouse IgG2a R-PE (Mouse IgG2a; Caltag), R-PE (Mouse IgG2a: CTkb), and FITC Rat IgG2b Isotype Control (eBioscience). Red blood cell lysis buffer reagents contained 8.3 g/L ammonium chloride in 0.01 M Tris–HCl buffer (pH 7.0). The cells were incubated with appropriately diluted Ab (1:100) for cell surface staining. Staining for FOXP3 was performed according to the manual of the mouse regulatory T cell staining set (eBioscience). Flow cytometry analysis was performed on a FACScan® (BD Biosciences, Shanghai, China) using CellQuest software.

Dex and IL-2 treatment
C57BL/6 mice were injected daily with Dex (5 mg/kg/day, i.p.) and/or IL-2 (300,000 IU/mouse/day, i.p.) for 3 days. The day following the last treatment, the mice were sacrificed and spleens were removed for subsequent experiments.

Mixed lymphocyte reaction
Cultures were set up in 96-well round-bottom plates (Costar, NY, USA) in a total volume of 200 μL. Cells were cultured in RPMI 1640 medium with 10% FBS, 10 mM HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin (GIBCO, Gaithersburg, USA). Fixed numbers of responder cells (separated from IL-2+Dex pre-treatment C57BL/6 mouse or from normal C57BL/6 mouse splenocytes) and irradiated allogeneic stimulator cells (splenocytes of BALB/c mice) (10^5 cells, respectively) were mixed. At various time points (0, 3, 6 days), CCK-8 reagents were added into the culture and then the OD values were measured.

GVHD model
BALB/c hosts were given total body irradiation (TBI; 500 cGy) from a Varian Clinic 600C X-ray source (Huashan Hospital, Shanghai, China) and injected with donor cells intraperitoneally within 24 h. All mice received 3–4 × 10^7 spleen cells. The survival and appearance of mice were monitored daily and body weight was measured weekly. The degree of systemic GVHD was assessed by a scoring system [37]. After 60–80 days post-transplantation, donor lineage-specific chimera in the transplanted animals was measured by staining host cells with anti-H-2Kβ and Y-chromosome polymerase chain reaction (PCR) analysis [38].

Assessment of GVHD
The degree of systemic GVHD was assessed by a scoring system that sums changes in five clinical parameters: weight loss, poor posture (hunching), activity, fur texture, and skin integrity (maximum index = 10) [37] (Table 1).

Y-chromosome PCR analysis
Genomic DNA was extracted from the host spleen cells using the Genomic DNA Purification Kit (TOYOBO Mag Extractor, Tokyo, Japan). Twenty-five microliters of the mixture was used per PCR reaction. Mouse SRY locus sequences on the Y chromosome in spleen cells were amplified by PCR with a specific pair of primers [38], 5'-CTGCTGTGAAACACACTAC-3' and 5'-GACTCCTCTGACTTCACTTG-3', resulting in a 722-bp genomic DNA fragment. Samples were heated to 94°C for 5 min and then amplified for 35 cycles at 94°C for 30 s, 59°C for 30 s, and 72°C for 1 min in 25 μl of PCR reaction mixtures. GADPH was amplified simultaneously as a control for PCR amplification with the primer pair 5'-CCAATTGTCTCCGTGATGATC-3' and 5'-GCTTCACCACCTTTGATGTC-3', resulting in a 660-bp genomic DNA fragment.
Histology analysis
Formalin-preserved liver, distal small bowel, and transverse large bowel were embedded in paraffin, and 5-μm thick sections were stained with hematoxylin and eosin for histological examination.

Statistical analysis
Comparisons of data from experimental groups and the control group, and comparisons of clinical scores between the various treatment groups were analyzed with a two-tailed student’s t-test using Graphpad Prism 4.0. Survival curves were plotted using Kaplan–Meier estimates. \( P, 0.05 \) was considered statistically significant.

Results

Combined Dex and IL-2 treatment selectively expands CD4\(^+\)CD25\(^+\)FOXP3\(^+\) Treg cells in vivo
We injected Dex (5 mg/kg/day) and/or IL-2 (300,000 IU/mouse/day) into normal C57BL/6 mice intraperitoneally for three consecutive days, with donor mice distributed into four groups: a Dex group with Dex treatment for 3 days, an IL-2 group with IL-2 treatment for 3 days, a Dex+IL-2 group with combined treatment for 3 days, and a control group of untreated mice. The percentages of CD4\(^+\)CD25\(^+\) donor spleen T cells and CD25\(^+\)FOXP3\(^+\) T cells (CD4 gated) were the highest in the Dex+IL-2 group [Fig. 1A,B].

The ratio of CD4\(^+\)CD25\(^+\) T cells to CD4\(^+\)CD25\(^-\) in donor spleens was evaluated, and it was found that combined treatment with Dex and IL-2 led to a 3-fold expansion of the CD25\(^+\)/CD25\(^-\) ratio compared with the control group. The IL-2 or Dex group also had higher CD25\(^+\)/CD25\(^-\) ratio than the control group, but either was lower than that of the Dex+IL-2 group [Fig. 1C]. The ratio in the Dex+IL-2 group was significantly higher than it was in the other groups (\( P < 0.05 \)) [Fig. 1D].

To demonstrate whether the Treg cells expanded by IL-2 and Dex had an inhibitory effect in mixed lymphocyte reactions (MLRs), we measured the OD values of the IL-2+Dex group and the negative control (NC) group at day 0, 3, and 6. It was shown that cell growth in the IL-2+Dex group was slower than that in the NC group (Fig. 2).

These results indicated that combined treatment with Dex and IL-2 markedly increased the proportion of Treg cells in the total splenic cells of the donors.

Effect of combined Dex and IL-2 treatment on Treg cells in a murine GVHD model
It was found that Dex+IL-2-treated donor grafts suppressed lethal aGVHD after MHC-unmatched allogeneic transplantation. Mice that received untreated donor grafts showed lethal aGVHD with the typical symptoms affecting weight, posture, activity, and fur texture [37]. Unexpectedly, recipients that received cells from Dex-treated donors showed long-term survival without GVHD symptoms. However, we failed to detect donor chimerism, indicating a failure in donor cell engraftment, so the survival and incidence for the Dex group were excluded from the statistics.

All control group mice were dead by 20 days post-transplantation with Grade 2 GVHD symptoms. The IL-2 group showed severe GVHD as well, with mortality beginning on day +10 and continuing until all were dead by 2 weeks after the transplantation. In contrast, >70% of the Dex+IL-2 group recipients survived this

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss (%)</td>
<td>&lt;10</td>
<td>10–25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Posture</td>
<td>Normal</td>
<td>Hunching noted</td>
<td>Severe hunching</td>
</tr>
<tr>
<td></td>
<td></td>
<td>only at rest</td>
<td>impairs movement</td>
</tr>
<tr>
<td>Activity</td>
<td>Normal</td>
<td>Mild to moderately decreased</td>
<td>Stationary unless stimulated</td>
</tr>
<tr>
<td>Fur texture</td>
<td>Normal</td>
<td>Mild to moderate</td>
<td>Severe ruffling/poor grooming</td>
</tr>
<tr>
<td>Skin integrity</td>
<td>Normal</td>
<td>Scaling of paws/tail</td>
<td>Obvious areas of denuded skin</td>
</tr>
</tbody>
</table>

Table 1 Measurement of aGVHD
period, and the median survival time was >60 day (vs. control group, 12 day, \( P < 0.001 \), Fig. 3). Transplantation of Dex+IL-2 pre-treated donor grafts resulted in the long-term survival of 52.9% (vs. 0% in the control group, \( P < 0.001 \)) of the recipient mice. Clinical GVHD was also quantified using a clinical scoring system. The average clinical scores for the control group, IL-2 group, and Dex+IL-2 group 14 days post-transplantation were 7.7 (\( n = 17 \)), 10 (dead, \( n = 4 \)), and 4.3 (\( n = 17 \)), respectively. Control animals developed severe GVHD (clinical scores > 5), which progressed over the observation period, whereas animals with Dex/IL-2 pre-treated donors had only moderate GVHD (clinical scores ranging from 3 to 5) that remained stable beyond 60 days. These results demonstrated the suppressive function of Dex/IL-2 pre-treated donor grafts both in terms of survival time and in the clinical appearance of GVHD. We also measured liver and bowel pathology in mice with and without aGVHD, as shown in Fig. 4.

Figure 1 IL-2 combined with dexamethasone can up-regulate Treg cells with immune inhibitory effect  Mice were treated daily with Dex, IL-2, or a combination of both for 3 days. One day after the last treatment, (A) the percentage of CD4<sup>+</sup>CD25<sup>+</sup> T cells in donor spleens was analyzed by flow cytometry. (B) The percentage of CD25<sup>+</sup>FOXP3<sup>+</sup> T cells in donor spleens was analyzed by flow cytometry. The percentage of CD25<sup>+</sup>FOXP3<sup>+</sup> T cells was analyzed by gating on the CD4 population. (C) Composition of the CD4<sup>+</sup> T cell population in the spleen of C57BL/6 donors. Flow cytometry assessed the CD25<sup>+</sup> and CD25<sup>-</sup> subpopulations. The ratio of CD4<sup>+</sup>CD25<sup>+</sup> cells to CD4<sup>+</sup>CD25<sup>-</sup> cells was calculated. (D) CD25<sup>+</sup> vs. CD25<sup>-</sup> T cell composition of splenocytes from C57BL/6 donors (*\( P < 0.05 \)).
The incidence of lethal aGVHD increased in the IL-2 group; all recipients were dead 13 days after the transplantation. This was dependent on the administration of CD4⁺CD25⁺FOXP3⁺ cells from the donor. These CD4⁺CD25⁺FOXP3⁺ T cells were believed to be effector T cells activated by a high dose of IL-2.

Figure 2 The effect of Treg cells expanded by Dex+IL-2 on MLRs
A C57BL/6 donor received Dex+IL-2 pre-treatment or saline pre-treatment, and splenocytes were mixed with BALB/c splenocytes. Growth curves were calculated by CCK-8 (P < 0.05).

Figure 3 BALB/c recipients of C57BL/6 allogeneic lymphocyte transplantation with Dex+IL-2 pre-treated donor cells in vivo are rescued from lethal aGVHD Recipients received donor cells pre-treated in vivo with IL-2 alone (solid red line) or Dex+IL-2 (solid blue line). The control group consists of BALB/c mice (dashed line). Combined data from three independent experiments with 4–17 mice per group are shown. P < 0.001 (Dex+IL-2 vs. control).

Figure 4 BALB/c recipients of C57BL/6 allogeneic lymphocyte transplantation with Dex+IL-2 pre-treated donor cells have slight tissue damage Microscopy of the liver (A,B) and the small intestine (C,D) were viewed after allogeneic lymphocyte transplantation using 500 cGy of total body irradiation as the conditioning factor. (A) and (C) indicate the histology of the liver and small intestine of a mouse that died of aGVHD. The manifestations were diffuse cellular swelling, degeneration of hepatic parenchymal cells and complete damage of the mucous membrane gland of the small intestine, as compared with the tissue from the Dex+IL-2 group host (B and D). Magnification, 400×.
Surviving mice showed donor chimerism after allogeneic transplantation

We then looked for chimerism in the long-term surviving mice by H-2Kb flow cytometry [Fig. 5(A)] and PCR for the SRY locus [38] [Fig. 5(B)]. The Dex group was negative for the expression of H-2Kb and for the presence of the SRY DNA fragment as mentioned; this indicated failure of donor cell engraftment. The other long-term surviving mice showed partial H-2Kb expression in splenic cells [Fig. 5(A)] and we detected

Figure 5 Detection of donor-derived hematopoiesis in mice by flow cytometry and Y-chromosome-specific PCR

Splenic DNA was amplified with Y-chromosome-specific primers (the mouse SRY locus) and with GADPH-specific primers resulting in a 722-bp fragment and a 660-bp fragment, respectively. (A) The H-2Kb MHC class I alloantigen expression of surviving Dex+IL-2 group mice 60–80 days after transplantation as compared with mice without transplantation. H-2Kb expression was analyzed by flow cytometry. (B) Forty-five days post-transplantation, the four recipients from the Dex group were free of GVHD clinical symptoms and were negative for the Y-chromosome-specific PCR fragment; this indicated transplantation failure. (C) Results are presented in three categories: male C57BL/6 mice, female BALB/c mice without transplantation, and BALB/c mice after transplantation (Dex + IL-2 pre-treatment C57BL/6 splenocytes as donor cells).
a 722-bp SRY DNA fragment in the PCR analysis [Fig. 5(C)]. Thus, these mice survived lethal GVHD due to the pre-treatment of donor cells before transplantation and the persistence of donor T cells in the recipient.

Discussion

The immune system does indeed exhibit homeostatic organization and it must regulate itself to avert insufficient immunity while suppressing excessive responses [21]. Regulatory CD4⁺ T cells that express CD25 play a vital role in the maintenance of tolerance to self-Ag and are required for the induction of non-responsiveness to allo-Ag [39].

Investigators have found that graft Treg content may predict the risk of GVHD. Depletion of CD25⁺ cells from human peripheral blood mononuclear cells (PBMCs) significantly exacerbated xenogeneic (x)GVHD and accelerated its lethality in RAG2²⁻/⁻ γc²⁻/⁻ mice, while co-administration of Treg-enriched CD25⁺ cell fractions with antigens demonstrated significantly reduced the lethality of xGVHD [20]. Murine studies have used ex vivo expanded purified CD4⁺CD25⁺ T cells to suppress GVHD [23,40] and to investigate the effects of CD4⁺CD25⁺ suppression of the MLR of CD4⁺CD25⁻ T cells with recipient stimulator cells [14]. The expression of these regulatory molecules in grafts and recipient peripheral blood after SCT has also been examined to show that Treg content is correlated with the development and progression of GVHD [39,41]. In this study, we used in vivo expansion of donor Treg cells to suppress GVHD, skipping the isolation, purification, and ex vivo expansion process, to provide a novel therapeutic approach using an immune prophylaxis method. One out of 17 Dex⁺IL-2 group recipients developed a typical skin ulcer 45 days post-transplantation. This likely represented graft-versus-host reaction or light chronic GVHD. Treg infusion ahead of effector T cell treatment with a Treg/Teff ratio at almost physiological doses (1:10) allowed the persistence of effective GVL responses [15]. In our study, the Treg/Teff ratio was 1:2.33, and the clinical manifestations indicated that GVL persisted.

According to previous studies, we used IL-2 and Dex at previously reported optimum doses [34]. IL-2 has been used clinically to enhance T cell immunity in patients with AIDS or cancer, and blockade with Abs to IL-2R has been used to inhibit T cell responses to transplanted tissues [42]. However, later studies discovered that IL-2 was critical for the development and peripheral expansion of CD4⁺CD25⁺ regulatory T cells [18,42]. Tregs express all of the subunits that are required for a functional high-affinity IL-2R [42], including the IL-2R α-chain, which enhances the affinity between IL-2 and its receptor, and is negatively expressed by naive effector T cells before being stimulated by antigens. Thus, Treg cells have higher expansion efficiency through IL-2 than Teff cells. IL-2 promotes Treg cell growth and suppressor function in vitro [43–45]. Adoptive transfer of wild-type Treg cells to IL-2R−/− or STAT5−/− deficient mice prevents lymphoproliferation and lethal autoimmunity [46,47]. Thymic expression of IL-2R in IL-2R−/− mice reconstitutes the production of Treg cells and prevents lymphoproliferation and lethal autoimmunity [42,48]. Some studies have found that using IL-2 after transplantation induced aGVHD [49], and that blocking IL-2 signal transduction reduced the incidence of GVHD [50]. In contrast, other studies have found that IL-2 might have preventive and therapeutic effects on GVHD [51]. Thus, IL-2 has both immune stimulatory and inhibitory effects. We previously demonstrated that a low dose of IL-2 simultaneously inhibits naive T cell proliferation and differentiation to Th1 [52]. An additional study indicated that IL-2 rescues Treg cells, but not Teff cells, from Dex-mediated cell death [36]. As mentioned previously, CD4⁺CD25⁺ T cells express higher levels of GR and Bcl-2, and are more resistant to Dex-mediated cell death than CD4⁺CD25⁻ T cells. In comparing Treg cells to Teff cells, Tregs have higher expansion efficiency through IL-2 than Teff due to IL-2 α-chain expression and are more resistant to Dex-mediated cell death due to GR and Bcl-2 expression. We believe that there may be an intersection between the IL-2-induced proliferation pathway and the GC-induced apoptosis pathway, but this currently remains unclear.

In this study, co-administration of Dex and IL-2 selectively expanded Treg cells rather than Teff cells in vivo. Our data are consistent with the observations of Chen et al. [34]. The ratio of CD4⁺CD25⁺ to CD4⁺CD25⁻ cells increased from 0.14:1 to 0.43:1. Activated CD4⁺ effector T cells express a wide range of receptors (like CD25), and they are the same as those found on naturally occurring Treg. A unique transcription factor, FOXP3, was found to be specifically expressed in murine and human Treg cells. FOXP3 encodes a forkhead/winged-helix transcriptional repressor termed Scurfin that may be specifically expressed in CD4⁺CD25⁺ Treg cells, and which is associated with their development and function [17]. FOXP3 mRNA expression was significantly lower in PBMCs from
patients with either allogeneic GVHD or autologous GVHD compared with patients without GVHD [39]. Reavani [24] also found that a high CD4+FOXP3+ T cell count in the donor was associated with a reduced risk of GVHD, and the ratio of CD4+FOXP3+ T cells to CD4+CD25+FOXP3− T cells was significantly reduced in patients with GVHD, suggesting diminished control of effector T cells.

The mean percentage of CD4+CD25+FOXP3+ cells in the Dex+IL-2 group was the highest among all four groups, and it was 2-fold higher than that of the control group. The IL-2 group contained the most CD4+CD25+FOXP3− T cells, which are believed to be activated effector T cells induced by a high dose of IL-2.

In the future, it might be possible to engineer donor grafts that are less capable of causing GVHD by increasing Treg cells, while retaining T cell responses specific for both tumor antigens and pathogens.

In the previous study, freshly isolated CD4+CD25+ T cells from unprimed animals may have prevented recipients from developing lethal GVHD after MHC-unmatched allogeneic transplantation. The addition of the CD4+CD25+ Treg cells at a 1:1 ratio with CD4+CD25− T cells resulted in a >90% inhibition of the mixed leukocyte reaction and marked protection from lethal GVHD [14]. In this study, recipients of Dex+IL-2 pre-treated donor cells had a prolonged survival time (median > 60 day vs. 12 day for the control group) and a superior overall survival rate (52.9% vs. 0%) compared with animals transplanted with untreated donor cells. We were able to rescue more than half of the BALB/c recipients (9 out of 17) that were given cells from Dex+IL-2 pre-treated donors. However, there were some differences between the experimental GVHD models applied by Hoffmann et al. [14], and by us, including differences in the preparatory regimen (sublethal vs. lethal irradiation), and in the numbers and phenotype of the transplanted cells, which may vary well account for the different outcomes of the experiments.

Severe GVHD clinical symptoms occurred in BALB/c mice that received IL-2 group donor cells; this was associated with the highest level of CD4+CD25+FOXP3− T cells. The Dex group mice failed in donor cell engraftment and were negative for the presence of H-2Kb MHC class I and SRY-specific PCR fragments. Thus, treatment with Dex or IL-2 alone may not be the proper Treg expansion regimen.

Further studies are underway to examine the role of NK and other cells in GVHD, as well as to assess the post-transplantation GVL effect of Dex+IL-2 in vivo-expanded Treg cells in malignancy disease models.

In conclusion, co-stimulation with Dex and IL-2 selectively expanded the functional CD4+CD25+FOXP3+ T cell population in vivo, and grafts from donors pre-treated with Dex and IL-2 led to longer survival times of the recipients and suppressed the effects of GVHD after allogeneic transplantation. Thus, GVHD can be suppressed by the specific expansion of regulatory T cells in the donor with Dex and IL-2 co-treatment.

**Funding**

This work was supported by a grant from the Science and Technology Commission of Shanghai Municipality, China (No. 06DZ19013).

**References**


24 Rezvani K. High donor FOXP3-positive regulatory T cell (Treg) content is associated with a low risk of GVHD following HLA-matched allogeneic SCT. Blood 2006, 108: 1291–1297.


43 Stanzani M, CD25 expression on donor CD4+ or CD8+ T cells is associated with an increased risk for graft-versus-host disease after HLA-identical stem cell transplantation in humans. Blood 2004, 103: 1140–1146.


50 Harris DT, Sakiestewa D and Lyons G. Prevention of graft-versus-host disease (GVHD) by elimination of recipient-reactive donor T cells with recombinant toxins that target the interleukin 2 (IL-2) receptor. Bone Marrow Transplant 1999, 23: 137–144.
