Absence of inducible costimulator on alloreactive T cells reduces graft versus host disease and induces Th2 deviation

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Inducible costimulator (ICOS) is expressed on activated and memory T cells and is involved in the regulation of cytokine production. We studied the role of ICOS on alloreactive T cells in graft versus host disease (GVHD) and determined that ICOS expression was up-regulated on alloreactive T cells in recipients of an allogeneic hematopoietic stem cell transplantation (allo-HSCT) with GVHD. We compared ICOS−/− T cells with wild-type (WT) T cells in 2 GVHD models. In both models, recipients of ICOS−/− T cells demonstrated significantly less GVHD morbidity and mortality, which was associated with less intestinal and hepatic GVHD but increased cutaneous GVHD. In addition, recipients of ICOS−/− donor T cells displayed a slight decrease in graft versus leukemia (GVL) activity. Further analysis of alloreactive ICOS−/− T cells showed no defect in activation, proliferation, cytotoxicity, and target organ infiltration. Recipients of ICOS−/− T cells had decreased serum levels of interferon-γ (IFN-γ), while interleukin-4 (IL-4) and IL-10 levels were increased, suggesting that alloreactive ICOS−/− T cells are skewed toward T helper-2 (Th2) differentiation. These data suggest a novel role for ICOS in the regulation of Th1/Th2 development of activated T cells. In conclusion, alloreactive ICOS−/− donor T cells induce less GVHD due to a Th2 immune deviation while GVL activity is slightly diminished.

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative therapy for hematologic malignancies and certain benign hematopoietic disorders. Apart from the antitumor effect of the conditioning regimen, graft versus leukemia (GVL) activity by alloreactive donor T cells plays an important role in the prevention of posttransplantation tumor relapse. However, alloreactive donor T cells are also responsible for graft versus host disease (GVHD), a major cause of posttransplantation morbidity and mortality. Thus, a major challenge for allo-HSCT is to inhibit GVHD while maintaining the beneficial effects of T-cell–mediated GVL activity.

T cells require 2 signals for an effective immune response. The first signal is generated by the interaction of the T-cell receptor (TCR) with a cognate peptide: major histocompatibility complex (MHC) on the surface of an antigen-presenting cell (APC). T cells that are stimulated via the TCR alone fail to produce the appropriate cytokines, are unable to sustain proliferation, and will often undergo apoptosis. The second signal is a costimulatory signal required to prevent apoptosis and induce full activation and differentiation into effector and memory T cells. Because full T-cell activation requires 2 signals, blocking T-cell costimulatory molecules is a strategy utilized to induce tolerance in solid organ transplantation and may be useful in allo-HSCT to inhibit GVHD. Costimulatory blockade has been studied in allo-HSCT models to induce immune deviation or suppression after transplantation. Inducible costimulator (ICOS) is a relatively new member of B7 family involved in the costimulation of T cells. This costimulatory family also includes CD28, cytotoxic T-lymphocyte antigen-4 (CTLA-4), and programmed death-1 (PD-1). The most studied receptor is CD28, which is constitutively expressed on naïve T cells and regulates the threshold for T-cell activation and interleukin-2 (IL-2) production. ICOS is expressed upon T-cell activation and contributes to the production of effector cytokines including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-4, IL-5, and IL-10 but may have little impact on IL-2 production. ICOS shares 28% amino acid identity and 39% similarity with CD28 and contains several highly conserved motifs that are found in CD28. ICOS expression is restricted to lymphoid organs such as spleen, lymph nodes, and Peyer patches. Mice genetically deficient for ICOS have normal thymi and normal peripheral T-cell development. However, because ICOS signaling induces CD40L expression on T cells, ICOS−/− mice are deficient in germinal center formation and immunoglobulin (Ig) class switching. This phenotype can be rescued by in vivo administration of an antibody that stimulates CD40 signaling.

The ICOS ligand (ICOSL) is constitutively expressed on unstimulated B cells, splenic and peritoneal macrophages, and blood-derived dendritic cells. Thus, ICOSL expression may be induced in peripheral tissues during inflammatory processes.

Recently, a number of studies have found that ICOS is involved in allograft rejection and that blockade of ICOS can promote allograft
Guo et al.24 showed that treatment with a blocking anti–mouse ICOS antibody kindly provided by Dr Richard Flavell (Yale University School of Medicine, New Haven, CT) could inhibit or treat GVHD as well as promote bone marrow engraftment.25 In an attempt to better understand the effects of ICOS blockade in allo-HSCT recipients, we used ICOS blockade in allo-HSCT models for GVHD and GVL activity. In this study we show that recipients of alloreactive ICOS blockade T cells display T helper-2 (Th2) immune deviation and have significantly less GVHD, while GVL activity is less affected.

Materials and methods

Cell lines and tissue culture

A20 and P815 were obtained from the American Type Culture Collection (Manassas, VA). 3Dpp210 was kindly provided by J. Griffin (Dana Farber Cancer Institute, Boston, MA).26 A20-TGL was generated by transducing A20 with a retroviral vector containing a fusion reporter gene coding for HSV1-TK, enhanced green fluorescent protein (eGFP), and firefly luciferase27 (a kind gift from Dr Vladimir Ponomarev, Memorial Sloan-Kettering Cancer Center). After transduction individual clones with high eGFP expression were sorted into 96-well plates using a FACS Vantage cell sorter (Becton Dickinson, San Jose, CA). Tissue culture medium consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM l-glutamine.

Mice and allo-HSCT

Female C57BL/6 (B6) (H-2b), C3FeB6F1 (H-2b/k), BALB/c (H-2d), C57BL/6-Ly5.1 (B6-Ly5.1) (H-2b), and B6D2F1 (H-2b/d) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). C57BL6:ICOS-KO (ICOS-/-) mice were kindly provided by Dr Richard Flavell (Yale University School of Medicine, New Haven, CT).13 Mice used in allo-HSCT experiments were between 8 and 12 weeks of age. Bone marrow transplantation (BMT) protocols were approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee. Bone marrow cells (BM) were removed aseptically from femurs and tibias of donor mice and were T-cell depleted (TCD) by incubation with anti-Thy-1.2 for 40 minutes at 4°C followed by incubation with Low-TOX-M antibody and washed twice with FACS buffer. The stained cells were incubated for 30 minutes.

Assessment of GVHD

Mice were randomly assigned to treatment groups and were followed for changes in weight and GVHD status over a period of 12 weeks. Survival was monitored daily, and mice were individually weighed and scored for changes in weight and GVHD status over a period of 12 weeks. Survival was measured by histopathology for evidence of microscopic tumor by a veterinary pathologist (Dr Krista LePerle, Cornell University Medical College, New York, NY), and cause of death was subsequently determined. If no evidence of tumor was detected and the animals displayed signs of GVHD, the cause of death was established as GVHD. If tissues were lysed at autopsy the cause of death was labeled as not analyzed.

Bioluminescent imaging

Animals that received A20-TGL were given 150 mg/kg (intraperitoneally) of D-Luciferin (Xenogen, Alameda, CA). Fifteen minutes after injection, mice were anesthetized with isoflurane and placed into the Xenogen IVIS bioluminescence (BLI) imaging system (Xenogen) in a supine position and recorded for 5 minutes. Pseudo color images showing the whole body distribution of bioluminescent signal were superimposed on the conventional gray-scale photographs.

GVHD histopathologic analysis

GVHD target organ pathology for small and large bowel and liver was assessed in a blinded fashion. Formalin-preserved organs were paraffin embedded, sectioned, hematoxylin and eosin (H&E) stained, and scored with a semiquantitative scoring system. Bowel and livers were scored for 18 to 22 different parameters associated with GVHD as previously described,29 and skin was evaluated for the number of apoptotic cells per millimeter of epidermis as previously described.30

Flow cytometric analysis

Cells were washed in fluorescence-activated cell sorter (FACS) buffer (PBS with 0.5% bovine serum albumin [BSA] and 0.1% sodium azide), and 106 cells per milliliter were incubated for 20 minutes at 4°C with CD16/CD32 Fc receptor (FcR) block. Subsequently, cells were incubated for 20 minutes at 4°C with antibodies and washed twice with FACS buffer. The stained cells were resuspended in FACS buffer and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) with Cell Quest software or with Flowjo software (Treestar, San Carlos, CA). All of the following fluorochrome-labeled antibodies against murine antigens were obtained from BD PharMingen (San Diego, CA): rat IgG2a (LOU), Ly9.1 (30C7), rat IgG2a (LOU), rat IgG2b (A95-1), CD3 (145-2C11), CD44 (IM7), CD62L (MEL-14), ICOS (7E.17G9), TCRβ (H57-597), rat IgG2a (LOU), CD4 (RM4-5), CD8 (53-6.7), rat IgG2b (R35-95), CD44 (IM7), CD8 (53-6.7), and H-2Kb (AF6-88.5).

Intracellular cytokine staining

In vivo priming. Spleenic donor T cells were harvested from recipients of an allo-HSCT with GVHD (as previously described) on day 11 after transplantation. In vitro priming. Splenocytes were initially incubated in a mixed lymphocyte reaction (MLR) with irradiated (2000 cGy) C3FeB6F1 (allogeneic), C57BL6 (syngeneic), BALB/c (third-party control) stimulators at a 1:3 effector-stimulator ratio and cocultured for 7 days. Subsequently, cells were harvested and restimulated for 14 hours with either allogeneic or...
CFSE labeling

Cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) as previously described. Briefly, T cells were incubated with CFSE at a final concentration of 2.5 μM (Molecular Probes, Eugene, OR) in Hanks balanced salt solution (HBSS) at 37°C for 15 minutes. Cells were washed 3 times with HBSS before intravenous injection.

51Chromium release assay

Target cells (allogeneic P815 and syngeneic EL4) were labeled with 3.7 MBq chromium 51 (51Cr) at 3 × 10³/ml for 1 hour at 37°C and 5% CO2. Labeled targets were plated at 5 × 10³ cells per well in U-bottom plates (Costar, Cambridge, MA). Effector cells were prepared (see below) and added at various effector-target ratios and were incubated for 4 hours at 37°C and 5% CO2. Subsequently, 35 μL of supernatant was removed from each well and counted in a gamma counter to determine chromium release.

In vitro priming. Wild-type (WT) or ICOS−/− splenocytes were incubated in an MLR with irradiated allogeneic C3FeB6F1 stimulators for 5 days at a 1:2 effector-stimulator ratio.

In vivo priming. Splenic donor T cells were harvested from recipients of an allo-HSCT on day 14 after transplantation.

Enzyme-linked immunosorbent assay (ELISA)

In parallel experiments, blood from animals with GVHD and control animals was harvested and tested by ELISA, which was performed according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

Statistical analysis

Statistical analysis of thymic and splenic cellularity and proliferation assays were performed with the nonparametric unpaired Mann-Whitney U test, whereas the Mantel-Cox log-rank test was used for survival data. The statistical analysis performed to test whether a differential change occurred between treatment groups was the pairwise difference in the AUC between treatment groups, whereas the Mantel-Cox log-rank test was used for survival data. The statistical analysis performed to test whether a differential change occurred between treatment groups was the pairwise difference in the AUC between treatment groups while ICOS expression was up-regulated on allogeneic donor T cells. Donor T cells with high ICOS expression also demonstrated an activated phenotype (CD25+, CD44hi, and CD62L−; data not shown). We conclude that alloreactive donor T cells express high levels of ICOS during GVHD.

ICOS+/− donor T cells induce less GVHD morbidity and mortality after allo-HSCT

To assess the role of ICOS in the development of GVHD after allo-HSCT, we used 2 well-described MHC disparate murine T-cell allo-HSCT models and analyzed the contribution of ICOS expression during allo-HSCT and GVHD. The recipient's T cells were labeled with CFSE and transferred into a sublethally irradiated (750 cGy) syngeneic B6 or allogeneic C3FeB6F1 hosts. The data shown in Figure 1A represent 1 mouse each from 1 independent experiment (normal control, and the black histograms represent staining with anti-ICOS monoclonal antibody. A horizontal line represents the fluorescence intensity of negative controls for both isotype-stained and unstained cells. T cells were stained for donor T-cell origin and CD4 and CD8 expression. A horizontal line represents the fluorescence intensity of negative controls for both isotype-stained and unstained cells. T cells were stained for donor T-cell origin and CD4 and CD8 expression.
allo-HSC models: B6→C3FeB6F1 and B6→BALB/c. In both models, lethally irradiated recipients received TCD allo-HSCs from WT B6 mice along with either WT or ICOS−/− T cells. We found in both models that recipients of ICOS−/− T cells developed significantly less GVHD morbidity and mortality (Figure 2).

**Traffic of ICOS−/− T cells to GVHD target organs is intact while tissue damage is decreased**

To further assess the role of ICOS in GVHD, we performed histopathologic analysis of specific target organs (Figure 3A). Tissues were harvested on day 14 (Figure 3A) or day 21 (Figure 3B-C) from lethally irradiated C3FeB6F1 mice that received TCD allo-HSC and either WT or ICOS−/− T cells. Using a semiquantitative scoring system, recipients of ICOS−/− T cells showed significantly less histopathologic damage in liver, small bowel, and large bowel (Figure 3A), while skin pathology demonstrated increased damage in the ICOS−/− T-cell recipients at day 21 (Figure 3B-C) and day 14 (data not shown). Contrary to GVHD in gut, liver, and skin, there was no significant difference in thymic GVHD, which is associated with a decrease in overall thymic cellularity as well as a decrease in the percentage of CD4+CD8+ thymocytes (Figure 3D). These results indicate that alloreactive ICOS−/− T cells generate less GVHD-associated liver and intestinal damage but more GVHD-associated skin damage. Lack of intestinal GVHD with severe skin GVHD has been described in Th2-mediated GVHD where signal transducer and activator of transcription-4 (STAT-4)−/− alloreactive T cells were infused in the allograft.

We then studied if protection from liver and intestinal damage in recipients of ICOS−/− T cells was due to a defect in their capacity to infiltrate target organs. We determined the absolute numbers of donor T cells during GVHD in liver and small intestine (Figure 3E-F) and detected no differences between recipients of ICOS−/− T cells versus WT T cells. These results indicate that alloreactive ICOS−/− T cells generate less GVHD-associated liver and intestinal damage but are able to infiltrate these target organs to the same extent as WT T cells.

**Alloreactive ICOS−/− T cells have intact activation, proliferation, and cytotoxicity**

The diminished GVHD activity of alloreactive ICOS−/− T cells could be due to an intrinsic defect in activation, proliferation, or cytotoxicity. To determine the capacity of ICOS−/− T cells to undergo alloreactive proliferation in vivo, CFSE-labeled T cells were transferred into an irradiated allogeneic host (B6→C3FeB6F1), and proliferation kinetics were compared with those of CFSE-labeled WT T cells. There was no significant difference in proliferation kinetics of ICOS−/− versus WT T cells. In some experiments both CD4+ and CD8+ ICOS−/− T cells showed a mild increase in the percentage of fast proliferative, alloreactive cells (Figure 4A). Also, activation of ICOS−/− T cells, determined by CD44 up-regulation, was comparable to WT T-cell activation (Figure 4B), indicating that alloreactive ICOS−/− T cells have intact activation and proliferation kinetics. We confirmed these data by examining the number of activated (CD44hi) donor T cells in spleens of allo-HSCT recipients with GVHD (Figure 4C). Again, no significant differences were found in both disparate and syngeneic models.
showed no detectable IFN-γ production by ICOS−/− T cells. Supernatants of T cells stimulated with allogeneic C3FeB6F1 splenocytes showed increased levels of IL-10 when comparing ICOS−/− T cells with WT T cells (WT = 14.68 ± 6.85 pg/mL; ICOS−/− = 42.34 ± 13.99 pg/mL; P = .04; ± represents SEM).

We also found that intracellular IFN-γ production was more than 3-fold decreased in CD4+ and 6-fold in CD8+ ICOS−/− donor T cells compared with WT T cells (Figure 5C). These data suggest that ICOS deficiency in alloreactive T cells can result in a Th2 immune deviation with decreased IFN-γ production and increased IL-4 and IL-10 production.

ICOS−/− donor T cells display intact GVL activity

To assess the effects of ICOS deficiency on the GVL activity of alloreactive T cells, we performed experiments in 2 well-characterized GVHD/GVL models: B6→B6D2F1 with P815 mastocytoma and B6→BALB/c with A20 lymphoma (Figure 6; Table 1). In the B6→B6D2F1 experiments (Figure 6A) a low dose of ICOS−/− T cells (0.5 × 10⁶) was used to decrease GVHD mortality and allow for a better measurement of GVL activity. Indeed, we observed no or minimal mortality in recipients of WT or ICOS−/− T cells without tumor challenge. Mortality in recipients that received TCD alloHSCs with T cells and P815 was significantly delayed compared with recipients of TCD alloHSCs and P815, but no differences were observed between recipients of WT versus ICOS−/− T cells. These results suggested that ICOS−/− T cells had intact GVL activity compared with WT T cells. Similar results were obtained in a second GVHD/GVL model (B6→BALB/c) with A20. In the first set of experiments (Figure 6B) no differences were noted in the rate of mortality comparing recipients of WT versus ICOS−/− T cells when challenged with A20 cells. In a second experiment, mice were challenged with A20-TGL to measure tumor burden by BLI (Figure 6C-D). Mice that did not receive T cells died early

Th2 immune deviation in recipients of ICOS−/− T cells

Previous studies have demonstrated that ICOS plays a role in the regulation of effector cytokine production by activated and memory T cells. Thus, protection from GVHD damage in recipients of ICOS−/− T cells could be due to differences in Th1/Th2 cytokine profiles. To address this question, cytokine levels in sera from allo-HSC recipients were tested by enzyme-linked immunosorbent assay (ELISA) (Figure 5). Sera were harvested at different days after transplantation in 6 independent experiments. At days 3, 8, and 10, there was a significant decrease of IFN-γ in sera of ICOS−/− T-cell recipients (Figure 5A). In contrast, there were increased serum levels of IL-10 (Figure 5A, left bottom panel) and IL-4 (Figure 5A, right bottom panel) compared with WT T-cell recipients. To further assess differences in IFN-γ production by donor WT versus ICOS−/− donor T cells, splenocytes from GVHD hosts were purified and stimulated in vitro with host antigen (Figure 5B). ELISA analysis from supernatants collected after 5 days of coculture showed no detectable IFN-γ when donor B6 WT or ICOS−/− T cells were cultured with B6 splenocytes (syn). When donor T cells were stimulated with C3FeB6F1 (allo) host splenocytes, again we found a significant decrease in IFN-γ production by ICOS−/− T cells. Supernatants of T cells stimulated with allogeneic C3FeB6F1 splenocytes showed increased levels of IL-10 when comparing ICOS−/− T cells with WT T cells (WT = 14.68 ± 6.85 pg/mL; ICOS−/− = 42.34 ± 13.99 pg/mL; P = .04; ± represents SEM).
Our study provides evidence, in a Th1-mediated disease, that ICOS blockade can result in immune deviation from Th1 to Th2 responses, which has not been previously described. This is in contrast to studies in allograft rejection, also a Th1-mediated disease, where ICOS inhibition ameliorated disease but did not induce Th2 deviation, because no Th2 cytokines were detected.21 Here we show that ICOS expression is induced during alloactivation of both CD4+ and CD8+ T cells and that alloreactive donor T cells in mice that have received an allo-HSCT and are undergoing GVHD also increase their ICOS expression. Recipients of an allo-HSCT containing ICOS−/− donor T cells had significantly less GVHD morbidity and mortality compared with mice that received WT donor T cells. ICOS deficiency in T cells had no effect on their in vivo activation, proliferation, and infiltration of target organs, but cytokine production was skewed in ICOS−/− T cells had no effect on their in vivo activation, proliferation, and mortality compared with mice that received WT donor T cells. ICOS-deficient alloreactive T cells in some cases displayed slightly decreased GVL activity.

**Discussion**

The role of ICOS in allo-HSCT was first studied in a graft versus host reaction (GVHR) models, where alloreactive T cells are adoptively transferred into an immunocompetent host.35 In this study, blockade of ICOS signaling accelerated acute GVHR.36 However, GVHR models are not comparable to true GVHD models for a variety of reasons. For example, differences between both models may be due to the lack of host myeloablative conditioning in the GVHR model. The conditioning regimen is critical for the earliest phase of acute GVHD and starts before donor cells are infused.1 Tissue damage of intestinal mucosa, liver, and other epithelial and endothelial surfaces activates host cells to secrete inflammatory cytokines. This cytokine cascade induces up-regulation of adhesion molecules and MHC antigens and enhances donor T-cell responses. The enhanced risk of GVHD after clinical HSCT is associated with extensive tissue damage, and this relationship between conditioning intensity, cytokine production, and GVHD severity has been supported in several animal models.29 Apart from the lack of myeloablative conditioning, GVHR models do not include the transfer of donor HSCs, host versus graft reactivity, posttransplantation hematopoietic reconstitution, and classic target organ GVHD. Morbidity and mortality in most GVHR models are due to donor T-cell reactivity against host hematopoietic cells resulting in pancytopenia. Therefore, the

### Table 1. Cause of death in allo-HSCT recipients challenged with tumor cells determined by bioluminescence and/or histopathologic analysis of liver and spleen

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N/A indicates not analyzed due to tissue necrosis.
pathophysiology of GVHR is significantly different from GVHD, and it is difficult to extrapolate findings in GVHR models to GVHD models and clinical GVHD. Moreover, the use of an inhibitory reagent in the GVHR models instead of ICOS-deficient donor T cells does not discriminate whether ICOS blockade impacts on host or donor-derived T cells.

Because Th1 inflammatory cytokines (like IFN-γ) play an important role in the cytokine cascade during the pathogenesis of GVHD as demonstrated by Ferrara and colleagues,37,39 a Th1 to Th2 shift in the initial response of donor T cells alleviates GVHD38,40 and preserves GVL activity.41 However, some studies show that while Th2 skewing increases survival, Th2 cells are required for the induction of hepatic and severe skin GVHD.34 Therefore, a bias toward Th2 cytokine production by ICOS−/− T cells may explain why in our model intestinal GVHD is decreased, resulting in an overall decrease in GVHD mortality, while skin GVHD is increased.

ICOS stimulation was initially thought to induce Th2 differentiation because blockade of ICOS signaling inhibited in vitro and in vivo Th2 differentiation.42-44 However, other in vivo models for Th1-mediated disorders, including allograft rejection,21,22,24 experimental autoimmune encephalomyelitis (EAE),45,46 and the Th1/Th2 model of collagen-induced arthritis,47 have demonstrated that blockade of ICOS/ICOSL interaction ameliorated disease. In most models of solid organ transplantation, blockade of ICOS/ICOSL signaling prevented or delayed graft rejection.24,48,49 Conflicting results arise when ICOS/ICOSL blocking reagents are administered at different phases of the immune response. Experiments in the EAE model show that blockade of ICOS/ICOSL during the priming phase exacerbates clinical symptoms; however, if blocking reagents are administered during the effector phase of the immune response, the clinical symptoms are abrogated.46 Similarly, in GVHR, blockade of ICOS signaling increased acute GVHR, while the same treatment abrogated chronic GVHR.36 Still, in most models of solid organ transplantation, blockade of ICOS/ICOSL signaling prevents or delays graft rejection.24,48,49

Experiments testing heart allograft survival in ICOS−/− mice showed similar results as using blocking reagents and demonstrated that lack of ICOS signaling increases heart allograft survival and reduced chronic rejection.24 In these experiments, lack of ICOS signaling resulted in a decrease in IFN-γ expression, similar to our results and to previous studies.15,45,47 Still, the role of ICOS in IFN-γ production is controversial. While most studies demonstrate that ICOS stimulation increases IFN-γ production,6,18,44,50 blocking ICOS/ICOSL interaction does not always impair IFN-γ expression.44,46 This discrepancy extends to the capacity of ICOS−/− T cells to produce cytokines. For example, ICOS−/− mice show weak Th2 in vivo responses, but cytokine production and Th2-mediated Ig class switching is rescued by the use of a strong adjuvants in vivo.12-14 Thus, the inflammatory milieu in which ICOS−/− T-cell activation occurs will determine the extent and type of cytokines produced. Importantly, in vitro differentiation of ICOS−/− T cells into Th1 and Th2 subpopulations shows that once ICOS−/− T cells have differentiated, Th1 cells generate less IFN-γ while IL-10 production is intact. Differentiated ICOS−/− Th2 T cells generate equal or slightly higher amounts of IL-4 and IL-10 than differentiated WT T cells.12 Our data in allo-HSCT are consistent with this finding, where alloreactive ICOS−/− T cells, once differentiated into Th2 type T cells, produce lower levels of IFN-γ and higher levels of IL-4 and IL-10 than WT Th2 T cells. However, we cannot exclude that cytokine production of alloreactive ICOS−/− T cells may vary depending on differences in the conditioning regimen, cytokine milieu, strain combinations, and other factors in different GVHD models.

A recent study by Taylor et al23 used GVHD models and found that ICOS blockade by administration of anti-ICOS antibodies or using ICOS−/− donor T cells resulted in a significant delay and decrease in GVHD morbidity and mortality. Interestingly, a greater survival benefit by ICOS blockade was observed in the setting of an intact STAT6 (Th2) pathway, which is in agreement with our observation that ICOS blockade might operate by skewing toward Th2.25

Recent experiments have indicated that ICOS stimulation preferentially induces transendothelial migration and polarization in Th1 cells and blocking of ICOS signaling inhibits Th1 migration but has no impact on Th2 differentiated T cells.51 Because our data in allo-HSCT suggest ICOS−/− T cells become Th2 effectors, it is to be expected that equal amounts of lymphocytes will infiltrate target organs. Indeed, our results show no difference in the infiltration of WT versus ICOS−/− T into GVHD target organs.

Importantly, we found that the in vitro cytolytic activity of ICOS−/− T cells was not impaired but showed slightly less in vivo GVL activity. Our studies and those of others have demonstrated that perforin-mediated cytolytic activity is required for optimal GVL in this particular GVHD/GVLM model with Pr15 mastocytoma, even under conditions of Th2 polarization.45,52,53 However, because IFN-γ is required for optimal GVL activity in some GVHD/GVM models,54 we cannot exclude the possibility that inhibition of ICOS in these cases would interfere with GVL activity.

Current strategies to block ICOS/ICOSL interactions are being generated and have been effective in the treatment of murine models for EAE,46 solid organ transplantation,21,22,24 lupus inflammatory diseases,4,44 and collagen-induced arthritis.47 In conclusion, our results indicate that blocking ICOS/ICOSL signaling could be an effective therapeutic target for the abrogation of GVHD through Th2 deviation, whereas GVL activity is less affected.

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References

20. Dong C, Juedes AE, Temann UA, et al. ICOS co-
19. Hill GR, Crawford JM, Cooke KR, Brinson YS, 
17. Yoshinaga SK, Whorisky J, Khare SD, et al. T-cell co-stimulation through B7R1-1 and ICOS 
15. Gonzalo JA, Delaney T, Goodearl A, et al. The influence of Fas ligand and perforin cyto-
14. Table 1: T-cell migration/polarization of memory/effector 
12. Vardi Y, Ying ZL, Zhang CH. Two-sample tests for 
11. Okamoto N, Nukada Y, Tezuka K, Ohashi K, Mi-
8. Matsuno K, Kobata T, Aizawa Y, Yagita H, Okumura K. Granulocyte colony-stimulating factor-induced granulocyte colony-stimulating factor-allogeneic stem cell transplantation maintains graft-versus-
7. Finnerty HF, et al. Cutting edge: the related molecules CD28 and inducible costimula-
4. Table 1: T-cell migration/polarization of memory/effector 
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1. Okamoto N, Nukada Y, Tezuka K, Ohashi K, Mi-

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