PREVENTION OF GRAFT-VERSUS-HOST DISEASE
BY ANTI-IL-7Rα ANTIBODY

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RUNNING TITLE: Prevention of Graft-versus-Host Disease (GVHD) by Anti-IL-7Rα Antibody

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Abstract

Graft-versus-host disease (GVHD) continues to be a serious complication that limits the success of allogeneic bone marrow transplantation (BMT). Using IL-7 deficient murine models, we have previously shown that IL-7 is necessary for the pathogenesis of GVHD. In the present study, we determined whether GVHD could be prevented by antibody-mediated blockade of IL-7 receptor α (IL-7Rα) signaling. C57/BL6 (H2Kb) recipient mice were lethally irradiated and co-transplanted with T cell depleted (TCD) BM and lymph node (LN) cells from allogeneic BALB/c (H2Kd) donor mice. Following transplantation, the allogeneic BMT recipients were injected weekly with either anti-IL-7Rα antibody (100 µg/mouse/week) or PBS for 4 weeks. Anti-IL-7Rα antibody treatment significantly decreased GVHD-related morbidity and mortality, compared to placebo (30% to 80%). IL-7Rα blockade resulted in the reduction of donor CD4+ or CD8+ T cells in the periphery by day 30 post-transplantation. Paradoxically, the inhibition of GVHD by anti-IL-7Rα antibody treatment resulted in improved long-term thymic and immune function. Blockade of IL-7R by anti-IL-7Rα antibody resulted in elimination of alloreactive T cells, prevention of GVHD, and improvement of donor T cell reconstitution.
Introduction

Graft-versus-host disease (GVHD) continues to be a limiting factor in the use of clinical hematopoietic stem cell transplantation (HSCT). GVHD occurs when donor T cells recognize host antigenic disparities expressed on antigen presenting cells (APC), resulting in activation of alloreactive T cells and destruction of host tissues. Patients with GVHD develop a wide range of symptoms including skin rash, diarrhea, liver disease, erythema, weight loss which eventually result in death (1-7). Immunosuppressive drug treatment or mature T cell depleted bone marrow transplantation (TCD BMT) have been used as effective strategies to prevent GVHD (8, 9). However, these strategies can also lead to engraftment failure, a prolonged state of immune deficiency, and various types of opportunistic infections. Therefore, developing a therapeutic strategy to suppress GVHD without compromising the immune system will be ideal for allogeneic BMT recipients.

IL-7 and Kit ligand (KL, stem cell factor [SCF]), are the major lymphopoietic cytokines produced in the thymus and BM compartment (10-13). IL-7 induces proliferation, differentiation, and survival of immature T lymphocytes. During normal T cell development in the thymus, IL-7 produced by thymic epithelial cells (TECs) binds to the cognate IL-7 receptor (IL-7R). The IL-7R is comprised of IL-7Rα and common γ subunits and expressed on the surface of immature T lymphoid progenitor cells. Mutations of the IL-7, IL-7Rα, and γc genes result in defective thymopoiesis and impaired ability to produce T lymphocytes (14-18). Previously we and others have shown that administration of recombinant human IL-7 following histocompatible BMT in murine recipients corrects thymopoietic defects and enhances immune reconstitution,
further suggesting the importance of IL-7 in development of T lymphocytes (19). Besides its thymopoietic effects, IL-7 also promotes expansion and survival of mature naïve and memory CD4⁺ and CD8⁺ T cells. Recent studies have shown that IL-7/IL-7R interactions in concert with low-affinity interactions between T cell receptors (TCR) and self-peptide ligands bound to MHC allow proliferation of mature T cells in the periphery (20-26). In addition, IL-7 enhances the survival of alloreactive donor T cells in allogeneic BMT recipients and plays a crucial role in the development and exacerbation of GVHD (27, 28, 30-32).

Based on the effects of IL-7 on mature T cells, we investigated whether GVHD could be prevented by a blockade of IL-7Rα with an anti-IL-7Rα monoclonal antibody. Similar to earlier experimental results that we obtained from the genetic model of IL-7 deficiency, we demonstrated that anti-IL-7Rα antibody treatment can successfully prevent GVHD by eliminating donor mature T cells (27). Paradoxically, anti-IL-7Rα antibody treatment did not impair donor-derived thymopoiesis even though IL-7 is critical for the development of T cells. These results indicate that anti-IL-7Rα antibody treatment may be beneficial for prevention of GVHD.
Materials and methods

Mice

Female C57BL/6J (H-2k\textsuperscript{b}, CD45.2), male B6.SJL (H-2k\textsuperscript{b}, CD45.1), male BALB/c (H-2k\textsuperscript{d} Thy 1.2), and male BALB/c (H-2k\textsuperscript{d} Thy 1.1) mice (aged 8 to 10 weeks) were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were kept in laminar flow cages with autoclaved food and acidified water. The protocol for maintaining animals pre and post-BMT was approved by the Childrens Hospital Los Angeles Research Institute Animal Care Committee (IACUC).

Bone marrow transplantation procedure

Female recipient H2K\textsuperscript{b} C57BL/6J mice were given two separate doses of radiation (700 cGy on day -1 and 600 cGy on day 0) as previously described (27). The BM from BALB/c (H2K\textsuperscript{d} Thy 1.1), BALB/c (H2K\textsuperscript{d} Thy 1.2), or B6.SJL donor mice were obtained by perfusion of the femur, and the lymph nodes (LN) from BALB/c (H2K\textsuperscript{d} Thy 1.2) were prepared by mincing of mesenteric, axillary, and inguinal lymph nodes, respectively. The donor BM cells were depleted for mature T lymphocytes by immunomagnetic depletion, using rat anti-mouse Thy 1, CD4, and CD8 monoclonal antibodies (Pharmingen, San Diego, CA) and sheep anti-rat antibodies conjugated to beads (Dynal, Great Neck, NY). Following irradiation of recipient mice, 1 x 10\textsuperscript{6} TCD BM and 4 x 10\textsuperscript{6} LN cells were transplanted into recipients via tail vein injection.

Administration of anti-IL-7R\textgreek{a} antibody
Anti-murine IL-7Rα antibody produced from the ST185 hybridoma clone (gift of Paul Kincade, University of Oklahoma) was purified using a Hi Trap Protein G HP antibody isolation kit (Amersham Biosciences, Uppsala Sweden). To test whether anti-IL-7Rα antibody treatment can prevent GVHD, allogeneic BMT plus LN recipients were subcutaneously injected weekly with either saline, nonspecific rat IgG (AbD Serotec, Raleigh, NC), or anti-IL-7Rα antibody (100 µg/mouse) from day 0 for 4 weeks. The clinical status of the BMT recipients was evaluated using a GVHD scoring system as previously described (28).

**Cell Proliferation Assay**

To test the anti-IL-7Rα antibody’s ability to block IL-7R signaling, 3H-thymidine incorporation assay was performed using the murine IL-7 dependent B cell line, 2E8 (ATCC, Manassas, VA). The 2E8 cell line was incubated with either freshly purified anti-IL-7Rα antibody (100 ng/ml) or non-specific IgG for 30 minutes and treated with rhIL-7 in various different concentrations for 24 hours to measure cell proliferation.

**Assessment of GVHD**

GVHD severity was assessed using the previously described clinical scoring system (28). Each BMT recipient was scored weekly for five parameters (weight loss, skin integrity, fur texture, mobility, and posture), using a scale of 0 to 2, with 0 for absent or normal, 1 for mildly abnormal, and 2 for severely abnormal. The GVHD clinical index was the sum of the scores for individual criteria.
**Histologic analyses**

Small intestine, skin, and thymic tissues were fixed in 10% formalin, embedded in paraffin, and cut into 5 µm thick sections for H&E staining. Tissue sections were examined for evidence of GVHD. Immunohistochemical staining of thymic sections were done as previously described (29). The thymus from normal and BMT recipient mice at day 30 post-BMT were embedded in Tissue-Tek OCT compound (SaKura, Torrence, CA) to make frozen section. Cryosections (5 mm) were immediately fixed with ice-cold acetone and incubated with 10% normal donkey serum (Sigma) for 10 minutes to block non-specific binding of antibodies. Thymic sections were stained with rabbit anti-mouse keratin 5 (Covance Research Products Inc., Berkeley, CA) and rat anti-keratin 8 (Troma-1, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) in 1% donkey serum overnight. As a negative control, normal rabbit or rat serum (Sigma) was used. FITC-conjugated donkey anti-rat IgG and Cy5- conjugated donkey anti-rabbit IgG (Jackson Laboratory) were used as secondary antibodies. Thymic tissue sections were viewed with a Leica DM RXA-RF-8 fluorescence microscope (Leica, Bannockburg, IL) using 5x/0.12 NA, HC Plan 10x/0.4NA, or HC Plan 20x/0.7 NA objective lenses for 50x, 100x, and 200x magnification, respectively. To capture images, a SkyVision-2/VDS-1300 12-bit digital camera (Applied Spectral Imaging, Carlsbad, Ca) with Easy FISH software (Migdal Ha'emek, Israel) was used.

**Immunophenotyping**

BMT recipients were sacrificed by CO₂ narcosis, and the thymus, spleen, and LN cells were removed. Single cell suspensions of each organ were prepared, and the total cell
numbers were determined. 1 x 10^5 cells were stained with conjugated antibodies directed against Thy1.1, Thy1.2, CD4, CD8, CD44, CD45.1, CD62L, H2K^d, or isotype control monoclonal antibodies (Pharmingen, San Diego, CA). After staining, cells were washed and analyzed on the FACS Calibur (Becton-Dickinson, San Diego, CA). In some experiments, donor LN cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) prior to transplantation to measure the proliferation in vivo of the cells after transplant. Donor LN cell proliferation was assessed by measuring separate peaks of decreased intensity of CFSE fluorescence upon successive cell division by FACS analysis of the labeled donor CD4 or CD8 T cell population. When cell numbers were limiting, LN cells from multiple recipients in each experimental group were pooled for analyses.

**Sheep RBC (SRBC) Immunizations**

Normal and recipient mice were immunized with SRBC (Colorado Serum Company, Denver, CO) via IP injection at day 44 post-BMT, as previously described (19). After 2 weeks, peripheral blood was drawn for primary antibody responses against SRBC, and the mice were boosted with SRBC for secondary responses, which were measured one week later. The agglutination antibody titers in the serum were determined by serial dilution and incubation with SRBC in 96-well V-bottom microplates (Corning Inc, Corning, NY).

**Statistical analyses**
Analyses of survival rates were performed by Winstat Survival Analyses (A-Prompt Co, Whitehall, PA). Different immunophenotypic populations of cells following transplant were analyzed by 2-tailed t-test with unequal distributions. P-values that were less than or equal to 0.05 were considered statistically significant.


Results

Decreased IL-7 dependent proliferation of 2E8 cell line by anti-IL-7Rα antibody

Since our previous data demonstrated that proliferation and maintenance of donor-derived allogeneic mature T cells are reduced in the absence of IL-7, we investigated whether a blockade of IL-7R signaling by anti-IL-7Rα antibody treatment had similar effects (27). To determine whether the anti-IL-7Rα antibody purified from the ST185 hybridoma supernatant binds to the murine IL-7Rα, lymph node T cells from a BALB/c mouse were incubated in vitro with the anti-IL-7Rα antibody. The purified anti-IL-7Rα antibody bound to both CD4 and CD8 T cells expressing IL-7Rα (Figure 1A). Next we performed a cell proliferation assay using the IL-7 dependent B cell line, 2E8 to determine if binding of anti-IL-7Rα antibody blocked IL-7 dependent proliferation. After incubating the cell line in media that contains anti-IL-7Rα antibody (100 ng/ml), IL-7 was added and proliferative capacity was measured. Figure 1B shows that anti-IL-7Rα antibody can effectively prevent proliferation of the 2E8 cell line. These results demonstrate that anti-IL-7Rα antibody blocks IL-7R signaling and prevents IL-7 dependent cell proliferation in vitro.

Anti-IL-7Rα antibody treatment prevents GVHD related morbidity and mortality

To measure the efficacy of anti-IL-7Rα antibody treatment in the prevention of GVHD, lethally irradiated (1300 cGy) B6 recipient (H2Kb) mice were co-transplanted with 1 x 10^6 TCD BM cells and 4 x 10^6 LN T cells from allogeneic BALB/c mice (H2Kd). Following transplantation, either 100 µg of IL-7Rα antibody or saline was
intraperitionally injected into allogeneic BMT plus LN recipients once a week for 4 weeks. In congenic and allogeneic BMT recipients (without LN cell administration), no mortality was observed (Figure 2A). The survival rate of allogeneic BMT plus LN recipients treated with saline was approximately 20% by day 150 after transplantation. In contrast, anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients remained significantly higher (70%). Although some of the anti-IL-7Rα antibody treated animals died of GVHD in the first 61 days post-transplantation, no further deaths were observed after day 61.

To further measure the effects of anti-IL-7Rα antibody in preventing GVHD, a GVHD scoring system was used to evaluate the clinical status of transplanted mice for the first 30 days after BMT (28). The recipients of either congenic or allogeneic BMT showed no signs of GVHD-related morbidity (Figure 2B). In contrast, allogeneic BMT plus LN recipients treated with saline displayed significant weight loss and other evidence of GVHD. Similar to the BMT only recipients, the anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients had a significantly lower GVHD clinical score compared to saline treated allogeneic BMT plus LN recipients. These results suggest that IL-7Rα antibody treatment reduces GVHD-related morbidity and mortality.

**Prevention of GVHD-related tissue inflammation by anti-IL-7Rα antibody**

To assess the effects of anti-IL-7Rα antibody on GVHD directly, GVHD-target organs were analyzed on day 30 after transplantation. Sections from the skin and small intestine were stained with H&E and assessed for histologic damage. The tissue samples from
allogeneic BMT plus LN recipients treated with saline displayed histological evidence of GVHD. The skin samples showed lymphocytic infiltration, hyperkeratosis, and hair loss, and the intestines had villus blunting, lymphocytic infiltration, lamina propria inflammation, crypt destruction, and mucosal atrophy (Figure 3). In contrast, tissue samples from anti-IL-7Rα treated allogeneic BMT plus LN recipients showed no histological evidence of GVHD and looked similar to those from normal, allogeneic, or congenic BMT recipients. These data provide evidence that anti-IL-7Rα treatment can prevent GVHD-related tissue damage and inflammation.

**Decreased numbers of donor T cells in the periphery of anti-IL-7Rα antibody recipients**

Since anti-IL-7Rα antibody treatment prevented GVHD, we investigated the effects of anti-IL-7Rα antibody on the survival of donor T cells in vivo. Compared to saline treated allogeneic BMT plus LN recipients, the total number of donor-derived CD4 and CD8 T cells was significantly lower in the spleen and LN of anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients at day 10 and 30 post-transplantation (Figure 4A and B). To ensure that antibody treatment did not cause non-specific IgG effects, e.g., Fc receptor blockade, some allogeneic BMT plus LN recipients were also treated with nonspecific rat IgG antibody as a control. GVHD lethality was similar between PBS and control IgG treated recipients (data not shown). The number of donor-derived T cells from the IgG treated group was not decreased when compared to saline treated recipients, and the control IgG group had significantly higher numbers of donor CD4 and CD8 T
lymphocytes in the LN than anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients (Supplemental Fig. 1). In addition, the kinetics of the reduction of allogeneic donor T cells in the peripheral blood of anti-IL-7Rα antibody treated recipients at day 10, 20, and 30 in these experiments showed that the reduction of donor T cell numbers is progressive over the first 20-30 days post-transplant (Supplemental Fig. 2).

To clarify the source of the T cells observed early after transplant, allogeneic TCD H2Kd Thy1.1+ BM cells were co-transplanted with H2Kd Thy1.2+ LN cells to distinguish BM-derived thymic emigrants from adoptively transferred T lymphocytes. BM derived allogeneic T lymphocytes (Thy 1.1) at day 10 were almost undetectable in the LN of allogeneic BMT plus LN recipients. In contrast, most of the donor-derived T lymphocytes detected in the LN were adoptively transferred LN-derived T lymphocytes expressing Thy 1.2 (Supplemental Figure 3). Therefore, early after transplant, almost all of the analyzable T cells are derived from the co-transplanted LN cells.

Although anti-IL-7Rα antibody prevents cell proliferation of the 2E8 IL-7 dependent cell line in vitro (Figure 1), the mechanism responsible for the decreased numbers of allogeneic donor T cells in vivo remained unclear. Therefore, we investigated whether inhibition of donor T cell expansion by anti-IL-7Rα antibody treatment contributes to lower numbers of CD4 and CD8 T cells in the periphery. CFSE-labeled LN cells from BALB/c donor mice were transplanted into lethally irradiated B6 recipients, treated with either anti-IL-7Rα antibody or saline. At day 10, donor-derived T cells in the lymph nodes of the recipient mice were analyzed by gating on H2k\textsuperscript{d}-positive donor T cells
Our data demonstrate that anti-IL-7Rα antibody treatment did not completely block proliferation of donor T cells. However, we observed that the frequency of proliferating donor T cells at day 10 in the presence of anti-IL-7Rα antibody was lower than that of saline treated recipients. The reduction of donor spleen and LN T cell numbers in anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients is consistent with the lower incidence of GVHD-related mortality we observed (Figure 2A).

**Anti-IL-7Rα antibody treatment results in improved GVHD-related thymic atrophy**

TECs are targets of GVHD in both clinical and experimental allogeneic BMT (33-35). Alloreactive donor T cells are known to damage the host thymic microenvironment. Since anti-IL-7Rα antibody treatment resulted in reduction of donor derived T cells in the periphery, we investigated whether the thymic architecture from the anti-IL-7Rα antibody treated recipients was also protected. The relative size of the thymus from saline treated allogeneic BMT plus LN recipients was much smaller than that of the normal thymus and displayed no demarcation between cortical and medullary regions (Figure 6A and data not shown). In contrast, the thymic histology from the anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients did not appear to be different from those of normal, congenic or allogeneic BMT recipients. Based on the histological analyses, we determined whether the TECs of the anti-IL-7Rα antibody treated recipients were normal. Thymic cortical and medullary epithelial cells have been well characterized by differential expression of cytokeratins (K5⁻ K8⁺ for cortical and K5⁺ K8⁻ for medullary) (36). Corresponding to the H&E data, the thymus from saline treated
allogeneic BMT plus LN recipients displayed abnormal cortical and medullary keratin expression patterns (Figure 6B). Both K5−K8+ cortical and K5+K8− medullary epithelial cells were scattered throughout the thymus, and the absence of distinctive cortico-medullary junctions was observed. In contrast, the thymus from anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients displayed normal cytokeratin expression patterns throughout the thymus, resembling normal and BMT only recipients.

The thymic tissue damage in saline treated allogeneic BMT plus LN recipients suggests that donor-derived T cell development might be also impaired. Therefore, the recipient thymus was analyzed for the relative distributions of donor thymic subpopulations at day 30 post-BMT. Shown in Figure 6C are representative flow cytometric analyses of the thymus from BMT recipients. The frequency of CD4+CD8+ double positive (DP) cells were greatly diminished, whereas the CD4+CD8− and CD4−CD8+ single positive (SP) cells were increased in saline treated allogeneic BMT plus LN recipients when compared to those from congenic or allogeneic BMT recipients. In contrast, the relative frequencies of the donor-derived thymocyte subpopulations in anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients were similar to those seen in normal mice or BMT only recipients. Correspondingly, the number of donor-derived thymocytes from anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients was significantly higher than saline treated allogeneic BMT plus LN recipients (Figure 6D).

To investigate whether the improvement of donor-derived thymopoiesis following anti-IL-7Rα antibody treatment is solely caused by the suppression of alloreactivity rather
than stimulatory or beneficial effects of anti-IL-7Rα antibody, we performed congenic TCD BMT (B6.SJL [H2Kb CD45.1+] donors) into lethally irradiated B6 recipients (H2Kb CD45.2+) and subcutaneously injected weekly them with either anti-IL-7Rα antibody or nonspecific rat IgG (100µg/mouse) for 4 weeks. At day 30 post-BMT, we observed that there is no beneficial effect in congenic hosts treated with anti-IL-7Rα antibody when compared to nonspecific rat IgG treated congenic recipients. (Supplemental Figure 4). These results demonstrate that anti-IL-7Rα antibody treatment not only prevents GVHD, but also improves donor-derived thymopoiesis by suppression of alloreactivity.

**Anti-IL-7Rα antibody treatment improved development of T cell dependent antibody responsiveness**

Since anti-IL-7Rα antibody treatment preserved donor-derived thymopoiesis at day 30, we then examined functional T cell dependent immunity by immunizing the transplanted recipients with sheep red blood cells (SRBC). The transplanted mice were immunized with SRBC on day 44 and the secondary boost was given two weeks after primary immunization (day 58). Following immunization, we measured primary and secondary antibody responses directed against SRBC. The saline treated allogeneic BMT and LN transplant group had low anti-SRBC titers after primary and secondary immunization (Figure 7). In contrast, the anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients displayed significantly higher secondary titers than the saline treated allogeneic BMT plus LN recipients, and titers in the anti-IL-7Rα antibody treated recipients were comparable to those observed in the normal and BMT only recipients.
Anti-IL-7Rα antibody treatment preserves donor-derived T lymphocyte populations long-term after allogeneic BMT

Since improved thymic histology and antibody response to SRBC immunization suggest that immune reconstitution is improved in anti-IL7Rα antibody treated recipients, we further investigated whether anti-IL-7Rα antibody treatment preserves donor T lymphocytes beyond day 30 post-allogeneic BMT plus LN transplantation. At day 250, we observed that the thymic histology and relative distributions of donor thymic subpopulations from the anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients appeared normal (Supplemental Figure 5). In addition, the presence of naïve donor-derived (CD62L+ CD44−) T lymphocytes in the spleen at later dates indicates the persistence of thymopoiesis. Since all the thymocytes and leukocytes in the spleen (including T cells) are donor-derived, our data strongly suggest that anti-IL-7Rα antibody treatment does not affect engraftment or the ability of donor hematopoietic progenitor cells to give rise to T cells.
Discussion

Since IL-7 plays a critical role in mature T lymphocyte expansion and survival, we investigated whether a blockade of IL-7R signaling by anti-IL-7Rα antibody can prevent the development of GVHD in the allogeneic setting. We observed significantly lower incidence of GVHD-related mortality in allogeneic BMT plus LN recipients treated with anti-IL-7Rα antibody (30%). The surviving mice remained free of GVHD for up to 8 months, even after anti-IL-7Rα antibody treatment was discontinued. In contrast, 80% of saline treated allogeneic BMT plus LN recipients developed GVHD and died by day 50 post-transplantation. The overall GVHD clinical index score of recipients treated with anti-IL-7Rα antibody was also significantly lower than that of the saline treated allogeneic BMT plus LN recipients. The anti-IL-7Rα antibody treatment resulted in decreased numbers of donor-derived CD4⁺ and CD8⁺ T lymphocytes in the peripheral organs of transplanted recipients early after transplant (before day 30). Taken together, the result of anti-IL-7Rα antibody treatment is similar to previous B6.IL-7⁻/⁻ allogeneic recipients in which the absence of host IL-7 production resulted in prevention of GVHD (28).

Although IL-7 was initially thought to regulate T and B lymphopoiesis, IL-7 signaling is also important for the peripheral expansion and survival of mature T cells (20-26). Adoptive transfer of naive congenic T lymphocytes into sub-lethally irradiated IL-7⁻/⁻ recipients results in minimal expansion of donor T cells, which could be restored by IL-7 administration (24). In addition, IL-7 mediates homeostasis of CD4 and CD8 memory T cells in vivo (20-22). IL-7 signaling also promotes survival of activated allogeneic donor
T cells that are prone to undergo activation-induced cell death (AICD) in early stages of GVHD (27). A recent report by Zhang et al. demonstrated that persistence of GVHD is caused by donor memory T cells whose homeostatic survival is enhanced by IL-7 (32). Since increased circulating levels of endogenous IL-7 are correlated with lymphopenia, a blockade of IL-7R signaling on alloreactive T lymphocytes in the early phases of allogeneic BMT may prevent GVHD by promoting apoptosis and suppressing expansion of host-alloreactive donor T cells (26, 37-39). We think that the reduced numbers of donor-derived T cells in antibody treated animals are mainly due to IL-7Rα blockade function rather than via antibody mediated cell lysis. The anti-IL-7Rα antibody can effectively inhibit proliferation of the IL-7 dependent pre-B cell line 2E8 in the presence of IL-7, as previously reported by Kincade et al (40). Here, the kinetics of disappearance of peripheral donor T cells in the anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients was relatively slow compared to what would be expected if the antibody was directly mediating cell lysis. Interestingly, the observed kinetics were similar to the rate of disappearance of donor T cells in our previous allogeneic IL-7−/− recipient models (28). Therefore, our data suggests that disappearance of donor T cells is more likely caused by blockade of IL-7R signaling than by antibody mediated lysis.

Mice and humans with mutations in the IL-7Rα or IL-7 genes have defects in thymopoiesis and a severe combine immunodeficiency (SCID) phenotype due to lack of production of mature T cells (14-18). Blockade of IL-7R signaling by the anti-IL-7Rα antibody might have been expected to inhibit donor-derived T cell development in the recipient thymus and delay donor immune reconstitution. Instead, we observed that anti-
IL-7Rα antibody treated allogeneic BMT plus LN recipients had improved thymic structure and cellularity compared to saline treated allogeneic BMT plus LN treated recipients. Similar to the congenic and allogeneic BMT recipients, anti-IL-7Rα antibody treated recipients displayed normal thymic structure including cortical and medullary demarcation. Donor alloreactive T lymphocytes infiltrate and damage the host thymus in experimental GVHD models (33-35). We hypothesize that the magnitude of the thymic protective effects of the anti-IL-7Rα antibody treatment exceeds any potential inhibitory effect of IL-7R blockade in the thymus. In addition, we demonstrated that the improvement of donor-derived thymopoiesis by anti-IL-7Rα antibody treatment is related to suppression of GVHD rather than a thymic stimulatory effect of either control IgG or IL-7R blockade. This was shown in congenic TCD BMT recipients in which no difference in donor-derived thymopoiesis was observed following nonspecific IgG or anti-IL-7Rα antibody treatment. It is also possible that the thymus-blood barrier excluded anti-IL-7Rα antibody from entering the host thymus and blocking T cell development (41-43). For example, Ghobrial et al. demonstrated that the amount of anti-CD4 or CD8 antibody required for depletion of mature T cells in the periphery was 1000-fold less than that required to cross the thymus-blood barrier to deplete thymocytes (44).

Following SRBC immunization, we observed that the response to a neo-antigen in anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients was significantly greater than the saline treated allogeneic BMT plus LN recipients. It is likely that the improvement of T cell dependent humoral immunity in the anti-IL-7Rα treated recipients was caused by donor-derived naïve T lymphocytes generated from the recipient thymus.
Although the low number of donor T lymphocytes in the spleen and LN of anti-IL-7Rα antibody treated recipients was observed at day 30 post-allogeneic BMT plus LN transplantation, the subsequently improved secondary antibody responses to SRBC is likely to be due to the increased number of peripheral donor T lymphocytes seen after cessation of anti-IL-7R treatment.

Due to the diverse TCR repertoire, naïve T cells generated from the thymus have the ability to respond to a wide variety of foreign antigens more effectively than memory T cells with a limited antigenic repertoire (45). The protection of the host thymus against donor alloreactive T cells provided by anti-IL-7Rα treatment resulted in greater immunocompetence, as measured by significantly higher antibody titers directed against SRBC after immunization. Since IL-7Rα antibody treated allogeneic BMT plus LN recipients showed no signs of GVHD for up to 8 months, it is likely that donor T cells developed from the thymus are not self-reactive.

Even though conventional regimens such as TCD BMT or immunsuppressive therapies can effectively prevent GVHD, delayed immune reconstitution and suppressed immune responses can often lead to fatal infections. In addition, complete depletion of donor T cells increases the risk of graft rejection by residual host T lymphocytes. Here we have demonstrated that blocking of IL-7R signaling following allogeneic BMT plus LN prevented GVHD while permitting immune reconstitution. The question of whether anti-IL-7Rα antibody specifically targets the donor T cells responsible for causing GVHD while sparing T cells required for development of tolerance is under investigation (46).
Our data demonstrates that transient blockade of IL-7R signaling by anti-IL-7Rα antibody may have therapeutic potential for prevention of GVHD.

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Authorship

Contribution: Brile Chung designed the research, performed the research, analyzed data, and wrote the paper; Eric Dudl performed the research; Dullei Min performed the research; Lora Barsky analyzed the data; Nancy Smiley contributed reagents; and Kenneth Weinberg designed the research and wrote the paper.

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Figure 1. Blockade of IL-7R signaling by anti-IL-7Rα antibody can effectively prevent proliferation of an IL-7 dependent cell line.

Anti-murine IL-7Rα antibody purified from the ST185 hybridoma clone was tested for its ability to bind to IL-7Rα and block IL-7 signaling. (A) Lymph node CD4 and CD8 T cells from a BALB/c mouse were incubated in vitro with the anti-IL-7Rα antibody purified from the ST185 hybridoma supernatant. (B) In order to measure the effect of anti-IL-7Rα antibody on cell proliferation, the IL-7 dependent murine B cell line, 2E8 was incubated in serum free media with either non-specific IgG or the anti-IL-7Rα antibody (100 ng/ml) for 30 minutes before IL-7 stimulation (12.5 pg/ml). For a negative control, the 2E8 cell line was incubated in media without IL-7 and IL-7Rα antibody. Cell proliferation was measured by determining the rate of ³H thymidine incorporation into DNA for 24 hrs after stimulation with or without IL-7. Asterisk indicates significant differences (p<0.05) between PBS and anti-IL-7Rα antibody treated groups.

Figure 2. Anti-IL-7Rα antibody treatment can successfully prevent GVHD related mortality and morbidity following allogeneic BM transplantation.

Following 1300 cGy TBI, B6 recipient mice were transplanted with 1x10⁶ TCD BM and 4x10⁶ LN cells from allogeneic BALB/c donor mice and injected with either PBS or anti-IL-7Rα antibody (100 µg/mouse/once a week) subcutaneously for 4 weeks. As controls, 1x10⁶ TCD BM cells from either congenic B6.SJL (CD45.1) or allogenic BALB/c (H2Kd) donors were transplanted into lethally irradiated B6 recipients. (A) Survival of anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients is significantly greater than that of the PBS-treated allogeneic BMT plus LN recipients (p<0.005). Survival over
the 150 days after BMT is shown. (B) The severity of GVHD was evaluated by using a GVHD clinical grading system with scoring for five clinical criteria: percentage of weight loss, skin integrity, posture, mobility, and fur texture (31). Clinical signs were graded on a scale of 0 to 2, where 0 was absent, 1 was moderate, and 2 was severe, and the individual signs summed. Shown are GVHD clinical index scores at day 30 following BMT. Difference between anti-IL-7Rα antibody and PBS-treated allogeneic BMT plus LN recipients is p < 0.0002 (asterisk).

**Figure 3. Histological analysis of anti-IL-7Rα antibody treated allogeneic recipients.**

Skin and small intestine tissues from the recipients was analyzed at day 30 following BMT. Representative tissue samples from each group of mice were stained with H&E. The skin and small intestine from the PBS-treated allogeneic BMT plus LN recipients showed evidence of GVHD with lymphocytic infiltration and inflammation. The tissue sections from anti-IL-7Rα antibody treated allogeneic BMT plus LN recipients had normal histology.

**Figure 4. Administration of anti-IL-7Rα antibody results in decreased numbers of donor T cells in the periphery following allogeneic BMT.**

Analyses of donor-derived peripheral CD4 and CD8 T cell numbers in anti-IL-7Rα antibody and PBS-treated B6 recipients were performed at day 10 and 30 following allogeneic BMT and LN cell transplantation. Shown are numbers of donor T lymphocytes at day 10 and day 30 in spleen (A) and LN (B). Asterisk indicates
significant differences between PBS and anti-IL-7Rα treated groups (spleen and LN at day 10 [p < 0.02]; spleen at day 30 [p < 0.02]).

**Figure 5. Administration of anti-IL-7Rα antibody results in decreased proliferation of donor-derived T cells.**

1x10^6 TCD BM and 4x10^6 CFSE-labeled LN cells from allogeneic BALB/c donors were transplanted into lethally irradiated B6 recipients. At day 10, the frequency of proliferating CFSE-labeled allogeneic donor-derived (H2k^d-positive) CD4 and CD8 T cells in LN (to the left of the indicated bar) of the anti-IL-7Rα antibody-treated allogeneic BMT and LN recipients was less than that of the PBS-treated recipients.

**Figure 6. Anti-IL-7Rα antibody treatment does not impair donor-derived thymopoiesis following allogeneic BMT.**

Both histological features and donor-derived cell analysis of the thymus from recipient mice were analyzed at day 30 post-BMT. Shown are representative thymuses from normal, PBS-treated congenic BM, PBS-treated allogeneic BM, PBS-treated allogeneic BMT plus LN, and anti-IL-7Rα antibody treated allogeneic BMT plus LN cell recipient mice. (A) Similar to normal and BMT-only recipients, the thymus from anti-IL-7Rα treated allogeneic BMT and LN cell recipients shows sharp demarcation between cortical (dense blue areas) and medullary (less dense blue areas) regions in H&E staining. Conversely, atrophic thymus from PBS-treated allogeneic BMT plus LN recipients displayed disorganized boundaries between cortex and medulla. (B) Thymic tissue
sections from transplanted recipients were stained with FITC-conjugated anti-K8 (green) and PE-conjugated anti-K5 (red) antibodies to characterize the cortical (K5⁻ K8⁺) and medullary (K5⁺ K8⁻) thymic epithelial cells (TEC). (C) Representative FACS analyses of CD4 and CD8 expression by donor-derived thymocytes at day 30 post-BMT. (D) The number of donor-derived thymocytes in BMT recipients at day 30. Difference between PBS and anti-IL-7Rα treated allogeneic recipients is p<0.05 (asterisk).

**Figure 7. Anti-IL-7Rα antibody treatment results in increased anti-SRBC titer following immunization.**

Anti-SRBC agglutinating titers were measured one week after the primary immunization (at day 44) and the secondary immunization (at day 58) post-BMT. Asterisk indicates significant differences between PBS and anti-IL-7Rα treated allogeneic BMT plus LN recipient groups (p<0.006).
References


45. Mackall CL, Bare CV, Granger LA, Sharrow SO, Titus JA, Gress RE. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. J Immunol. 1996; 156(12): 4609-16.

Figure 2A

![Graph showing survival (%) against days (post-transplant) for different groups: Congenic BMT (PBS) (N=10), Allogeneic BMT (PBS) (N=10), Allogeneic BMT + LN (Anti-IL-7Rα) (N=20), Allogeneic BMT + LN (PBS) (N=20). The graph indicates that the Allogeneic BMT + LN (Anti-IL-7Rα) group had a lower survival rate compared to the others.

Anti-IL-7Rα antibody Treatment

Figure 2B

![Bar chart showing GVHD score for different groups: Congenic BMT (PBS) (N=10), Allogeneic BMT (PBS) (N=10), Allogeneic BMT + LN (PBS) (N=10), Allogeneic BMT + LN (Anti-IL-7Rα) (N=10). The chart indicates that the Allogeneic BMT + LN (Anti-IL-7Rα) group had a significantly higher GVHD score compared to the other groups.]
Figure 3

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<th>Allogenic BMT + LN (PBS)</th>
<th>Allogenic BMT + LN (Anti-IL-7Rα)</th>
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Figure 4A

- **PBS (N=5)**
- **Anti-IL-7Rα (N=5)**

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</table>
Figure 4B

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Figure 5
Prevention of graft-versus-host disease by anti-IL-7Rα antibody

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