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Luteinizing Hormone-Releasing Hormone Enhances T Cell Recovery following Allogeneic Bone Marrow Transplantation

Gabrielle L. Goldberg,* Christopher G. King,† Rebecca A. Nejat,‡ David Y. Suh,‡ Odette M. Smith,* Jamison C. Bretz,‡ Robert M. Samstein,* Jarrod A. Dudakov,‡ Ann P. Chidgey,‡ Selina Chen-Kiang,§ Richard L. Boyd,§ and Marcel R. M. van den Brink3*

Posttransplant immunodeficiency, specifically a lack of T cell reconstitution, is a major complication of allogeneic bone marrow transplantation. This immunosuppression results in an increase in morbidity and mortality from infections and very likely contributes to relapse. In this study, we demonstrate that sex steroid ablation using leuprolide acetate, a luteinizing hormone-releasing hormone agonist (LHRHa), increases the number of lymphoid and myeloid progenitor cells in the bone marrow and developing thymocytes in the thymus. Although few differences are observed in the peripheral myeloid compartments, the enhanced thymic reconstitution following LHRHa treatment and allogeneic bone marrow transplantation leads to enhanced peripheral T cell recovery, predominantly in the naïve T cell compartment. This results in an increase in T cell function in vivo and in vitro. Graft-versus-host-disease is not exacerbated by LHRHa treatment and graft-versus-tumor activity is maintained. Because LHRHa allows for reversible (and temporary) sex steroid ablation, has a strong safety profile, and has been clinically approved for diseases such as prostate and breast cancer, this drug treatment represents a novel therapeutic approach to reversal of thymic atrophy and enhancement of immunity following immunosuppression. The Journal of Immunology, 2009, 182: 5846–5854.

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allogeneic bone marrow transplantation (BMT) is a potential therapeutic treatment for several hematological malignant diseases. Although this therapy increases overall patient survival, the ensuing immunosuppression, which is most severe in the T cell compartment, leads to opportunistic infections that are major contributors to posttransplant morbidity and mortality (1). Immune recovery is inversely proportional to age and is therefore of particular concern in older patients (2). The delay in T cell recovery in adults is mainly due to age-associated thymic atrophy and the concomitant decline in thymic export of naïve T cells (2).

Sex steroids have known roles in sexual dimorphism, differentiation, and development. However, sex steroids also affect most hematopoietic developmental stages as well as the function of mature immune cells. Little is known about the effect of androgens on early hematopoietic progenitors other than evidence that androgen receptors are expressed on adult lymphoid precursors and CD34+ human cord blood cells (3). The hematopoietic stem cell (HSC) number does not significantly differ between (estrogen receptor α or ERα−/− and ERα+/− mice. However, a population of Lin− c−kit+gh cells (the population that contains HSCs) is estrogen sensitive (4). Following estrogen treatment, this population is significantly decreased. The majority of the resistant cells have myeloid potential while lymphoid progenitor potential is dramatically decreased, suggesting that there is a population of cells within the early hematopoietic progenitor compartment that is both lymphoid committed and steroid sensitive (4). Myeloid progenitors in mice appeared unaffected by hormone treatment (4) while circulating monocytes and peritoneal macrophages are increased following estrogen administration (5). The treatment of mice with either androgens or estrogens results in a wide range of inhibitory effects on B cell development. Conversely, castration and ovariecctomy exert predominantly stimulatory effects (6, 7). Studies from our laboratories and others have shown that both surgical and chemical castration can reverse age-related thymic atrophy (8–14). Furthermore, we have shown that surgical castration enhances thymic and peripheral T cell reconstitution following antineoplastic therapy and in the settings of both autologous and allogeneic BMT (15–18). From a clinical perspective, it will be important to determine whether similar effects can be obtained using chemical castration.

Accordingly, in this present study, we have translated this into a clinically applicable model using the agonistic leuprolide acetate variant Lupron (TAP Pharmaceutical Products), a widely used luteinizing hormone-releasing hormone (LHRH) agonist. LHRH is a decapeptide, released in a pulsatile fashion by the hypothalamus. It
acts on the pituitary and controls the release of luteinizing hormone and follicle-stimulating hormone. When LHRH is released in a continuous fashion at sufficiently high levels (such as with leuprolide acetate treatment, prepared as a long-term slow release formulation), after an initial sex steroid surge, LHRH receptors are desensitized and there is a subsequent decrease in luteinizing hormone and follicle-stimulating hormone production, which in turn lead to a decrease in gonadal sex steroid production. The effect is prolonged castrate levels of both estrogen and testosterone for as long as LHRH is circulating. In addition to its chemical castration effects, LHRH may also directly affect cells in the thymus that express LHRH receptors.

This treatment is advantageous because chemical castration is reversible with few, if any, long-term side effects. Furthermore, LHRH agonist is in clinical use for the treatment of a variety of sex steroid-exacerbated disorders such as precocious puberty (19), prostate cancer (20), endometriosis (21), and breast cancer (22). Therefore, extensive information is available regarding its pharmacokinetics, dosing, toxicity, and efficacy.

In this study we treated recipients of murine allogeneic BMT with leuprolide acetate and analyzed bone marrow (BM), thymic, and splenic reconstitution. We found significant increases in hematopoietic precursors in the bone marrow and enhanced thymic and peripheral T cell reconstitution without exacerbation of graft-versus-host disease (GVHD) and with maintenance of graft-versus-tumor (GVT) activity.

Materials and Methods

Mice and BMT

B6.SJL (Ly5.1; H-2b) and BALB/c (H-2d) female mice (The Jackson Laboratory) were used between 8 and 12 wk of age. BMT protocols were approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee. BM cells were depleted of T cells by incubation with anti-Thy-1.2 for 40 min at 4°C and incubation with Low-TOX-M rabbit complement (Cedarlane Laboratories) for 40 min at 37°C. Splenic T cells (for GVHD experiments) were purified over a nylon wool column. Cells were transplanted by tail vein infusion (0.2-ml total volume) into lethally irradiated recipients on day 0. Recipients received 850 cGy total-body irradiation (137Cs source) as a split dose with 3 h between doses. Mice were housed in sterilized microisolator cages and received normal chow and autoclaved hyperchlorinated drinking water (pH 3.0). The P815 tumor cell line was obtained from American Type Culture Collection.

Sex steroid ablation

Lupron (leuprolide acetate), an LHRH agonist (TAP Pharmaceutical Products), was administered i.m. at a dose of 0.4 mg/mouse 28 days before BMT.

Flow cytometric analysis

BM cells, splenocytes, or thymocytes were washed in FACS buffer (PBS/2% BSA/0.1% azide) and 1–2 × 10^6 cells were incubated for 30 min.
at 4°C with primary Abs and CD16/CD32 FcR block, then washed twice with FACS buffer. Donor-derived cells were CD45.1/H11001. When necessary, cells were incubated with conjugated streptavidin for a further 30 min at 4°C. Cells were resuspended in FACS buffer and analyzed on an LSR2 flow cytometer with FACSDiva software (BD Biosciences).

Anti-CD3 and anti-CD28 T cell proliferation assay

Splenocytes (4 × 10⁵ cells/well) were stimulated with plate-bound anti-CD3 (145-2c11) and anti-CD28 (37.51; 0.5 g/ml as a final concentration of each) for 2 days. Cultures were pulsed during the final 18 h with 1 Ci/well [3H]thymidine and harvested in a Top Count Harvester (Packard Biosciences). Stimulation indices were calculated as the ratio of stimulated cells (cpm) over unstimulated cells (cpm).

Third-party MLR

For one-way MLRs, splenocytes from transplanted mice (2 × 10⁵ cells/well) were incubated, in 96-well plates, for 5 days with irradiated (2000 cGy) B10.BR splenocytes as stimulators (2 × 10⁵ cells/well). Two weeks before the in vitro assay, transplanted mice (in the vaccinated group) were injected i.p. with 10⁷ irradiated B10.BR splenocytes. Cultures were pulsed during the final 18 h with 1 μCi/well [3H]thymidine and DNA was harvested on a Top Count Harvester (Packard Biosciences).

ELISPOT for T cell-dependent B cell response

Transplanted mice were injected with 75 mg of 4-hydroxy-3-nitrophénylacetyl (NP)-(23)-chicken γ-globulin (CGG; Biosearch Technologies) precipitated in a 100 mg/250-ml solution of aluminum hydroxide adjuvant on day 35. Spleens were harvested on day 42 and an ELISPOT was used to quantify the number of plasma cells that were secreting NP-specific IgM or IgG at the single-cell level. 50 g/ml NIP-BSA was coated onto multiscreen-HA membrane plates (Millipore) overnight. Beginning with a 100-μl suspension of 10⁷ B cells/ml, 3-fold serial dilutions were then cultured on the precoated HA-multiscreen membrane plates for 3.5 h in complete RPMI 1640 at 37°C in a 5% CO₂ chamber. Bound IgG or IgM was detected by sequential incubation with biotinylated affinity-purified goat anti-mouse IgG or IgM (1/20,000 dilution in PBS, 0.1% Tween 20, and 5% FCS; Sigma-Aldrich) and peroxidase-conjugated streptavidin (1/1,000 dilution in PBS, 0.1% Tween 20, and 5% FCS; Vector Laboratories). Secreting cells were detected using 3-amino-9-ethylcarbazole (Sigma-Aldrich) and counted in duplicate.

Immunohistochemistry

All tissues were freshly dissected, placed in plastic trays, and covered with OCT (Tissue-Tek; Miles). These were snap frozen on a liquid nitrogen/isopentane slurry. Sections (6–10 μm) were cut on a cryostat, air dried, and fixed in acetone for 1 min. Slides were incubated with 30 μl of α-cytokeratin (DakoCytomation) for 15 min in a moist container at room temperature, washed in PBS with gentle agitation for 5 min, then incubated with 30 μl of anti-rabbit-Alexa Fluor 488 (Invitrogen), followed by washing.
Confocal microscopy

A Leica TCS SP2 AOB5 one- and two-photon laser-scanning inverted confocal microscope was used to view sections and to capture images.

GVHD

C57BL/6 T cell-depleted (TCD) BM cells (5 × 10⁶) and either 0.5 × 10⁶ or 2 × 10⁶ nylon wool-passed C57BL/6 T cells were injected i.v. into lethally irradiated (850 cGy) BALB/c recipients. The severity of GVHD was assessed with a clinical GVHD scoring system as first described by Cooke et al. (23). Briefly, ear-tagged animals in coded cages were individually scored every week for five clinical parameters on a scale from 0 to 2: weight loss, posture, activity, fur, and skin. Survival was monitored daily. Animals with scores of 5 or more were considered moribund and were humanely killed.

Assessment of GVT-P815 mastocytoma induction and assessment of tumor death vs death from GVHD

B6D2F1 recipients received 1 × 10³ P815 cells i.v. on day 0 of allogeneic BMT, as well as 5 × 10⁶ TCD BM cells and 1 × 10⁶ nylon wool-passed T cells, both of C57BL/6 origin. Survival was monitored daily and the cause of death after BMT was determined by necropsy.

Results

To determine whether chemical castration enhanced immune reconstitution following allogeneic BMT, male BALB/c mice were treated with leuprolide 28 days before lethal irradiation and i.v. injection of 5 × 10⁶ TCD B6 Ly5.1 BM cells. BM, thymus, and spleen were harvested and analyzed for differences in chimerism, total organ cellularity, BM precursor subsets, myeloid cells, thymocytes, T cells, and B cells.

We assessed donor/host chimerism in the BM, thymus, and spleen 14, 28, and 42 days after allogeneic BMT. Although at the earlier time points host cells were present in all three organs, by day 42, the vast majority of cells were of donor origin (Fig. 1, A–C). There is no significant difference in the donor/host chimerism when comparing control mice to those that received leuprolide.
donor-derived CLPs translate to a significant increase in donor-proliferate acetate group (Fig. 2C). Significantly increased 42 days after allogeneic BMT in the leu-
junction (Fig. 4). The reconstitution of myeloid progenitors was also assessed. Donor-derived common myeloid progenitors (lineageeGc-kit−CD34+FCγRlow), granulomonocytic progenitors (lineageeGc-kit−CD34+FCγRhigh), and megakaryocyte-erythrocyte progenitors (lineageeGc-kit−CD34+FCγRlow) were all significantly increased 42 days after allogeneic BMT in the leuprolide acetate group (Fig. 2C). The increases observed in donor-derived CLPs translate to a significant increase in donor-
derived B cell numbers in the BM 28 and 42 days after allogeneic BMT in the leuprolide acetate-treated group (Fig. 2D). Interestingly, the differences observed in the myeloid precursor subsets did not lead to significant differences in donor-derived myeloid cell numbers in the BM (Figs. 2, E–G), with the exception of an increase in donor-derived dendritic cells 28 days after allogeneic BMT (Fig. 2G).

Although changes in the BM only reach significance at later time points, thymic cellularity is significantly increased as early as 14 days after transplant in the leuprolide acetate-treated group and remain elevated through to day 42 (Fig. 1B). The majority of the differences observed are due to significant increases in donor-de-

derived double-positive (DP) thymocyte numbers in the leuprolide acetate-treated group (Fig. 3F). However, augmentation of thymic reconstitution can be observed as early as the triple-negative 1 (TN1; lineageeGCD44−CD25−) stage of thymocyte development (Fig. 3A). Donor-derived TN2-TN4 cell numbers are also significantly increased at different stages of reconstitution (Fig. 3, B–D); however, this does not result in a significant increase in total do-

ner-derived CDγ3−CD4−CD8− TN cell numbers at any of the time points analyzed (Fig. 3E). Donor-derived mature single-pos-

itive (SP) CD4+ and CD8+ thymocytes are significantly increased 42 days after BMT in the leuprolide acetate-treated group (Fig. 3, G and H).

To further analyze thymic reconstitution, thymus sections were stained for cytokeratin and the overall architecture of the thymus was analyzed. Fourteen days after BMT, the thymic architecture of leuprolide acetate-treated mice had clear cortical and medullary regions and a well-defined cortical medullary junction (Fig. 4A). However, in control recipients, the cortical and medullary regions appear to be less organized and the junc-
tion less obvious (Fig. 4A). This suggested that leuprolide acetate treatment can enhance restoration of thymic architecture. By day 2, both the control group and leuprolide acetate group had clear cortical and medullary areas (Fig. 4B) and by day 42 the thymus from both groups appear indistinguishable from un-

transplanted mice (Fig. 4C). The changes seen in the BM and thymus translated into a sig-

ificant difference in total splenic cellularity 42 days after BMT (Fig. 1C). At this time, donor-derived CD4+ splenic T cells of naive, central memory/activated, and effector memory phenotypes are all significantly increased (Fig. 5A), as are donor-derived naive and effector memory CD8+ T cells (Fig. 5B). A significant in-

crease in donor-derived B cells was also observed 28 days after allogeneic BMT and leuprolide acetate treatment (Fig. 5C). How-

ever, no significant differences were observed in the myeloid sub-

sets analyzed (Fig. 5, D–F).

To determine whether the increases in donor-derived T cells in the leuprolide acetate group translate into an increase in T cell function after allogeneic BMT, we performed a series of in vitro and in vivo T cell assays. Proliferation of an equal number of splenocytes from control and leuprolide acetate-treated mice was analyzed in response to anti-CD3/anti-CD28 stimulation. There was a significant increase in splenic T cell proliferation in the leuprolide acetate-treated group 28 days after allogeneic BMT (Fig. 6A).

When splenocytes from unvaccinated transplanted mice were analyzed in a third-party MLR 42 days after BMT, very little response was observed (Fig. 6B). To illicit a measurable re-

sponse, we vaccinated transplanted mice with irradiated TCD third-party (B10. BR) splenocytes 14 and 7 days before the MLR and detected a greater response with splenocytes from leuprolide acetate-treated recipients compared with control re-

cipients (Fig. 6B). Although there appears to be an increase in proliferation in the leuprolide acetate-treated group following

FIGURE 4. Thymic architecture is restored more rapidly in leuprolide acetate-treated mice following allogeneic BMT. Eight- to 12-week-old male BALB/c mice were lethally irradiated and transplanted with 5 × 106 B6.SJL (Ly5.1) TCD BM cells. Mice were injected with leuprolide acetate 28 days before BMT. A. Thymic sections of control and leuprolide acetate-
treated mice 14 days after allogeneic BMT. B. Thymic sections of control and leuprolide acetate-treated mice 28 days after allogeneic BMT. C. Thymic sections of control and leuprolide acetate-treated mice 42 days after allogeneic BMT. Thymic sections were stained with an anti-
cytokeratin Ab. Dashed line represents corticomedullary junction. C, Cortex; M, medulla.
allogeneic BMT, the percentage and number of IFN-γ-producing donor-derived CD4+ and CD8+ T cells was not significantly different in the two groups (Fig. 6C).

To assess whether T cell responses were augmented in vivo, specifically T cell-dependent B cell responses, mice were injected with hapten-conjugated CGG (NP23-CGG) 35 days after allogeneic BMT. On day 42, we harvested the spleens and performed an ELISPOT to analyze the number of Ag-specific plasma cells. The percentage and number of total plasma cells were significantly increased in the leuprolide acetate-treated group. The number of Ag-specific plasma cells was also increased, but this difference did not reach statistical significance (Fig. 6D).

Any treatment used to enhance immune reconstitution runs the risk of exacerbating GVHD. We therefore assessed the effect of leuprolide acetate on the severity of GVHD. Either 0.5 × 10^6 or 2 × 10^6 T cells were transferred with the allograft to induce GVHD. There was no significant difference between leuprolide acetate-treated and control mice, at either dose, with respect to percent survival (Fig. 6E). Furthermore, when the recipients were challenged with P815 mastocytoma cells, we found that GVT activity was maintained in the leuprolide acetate-treated recipients (Fig. 6E).

Discussion

In this study, we have moved into a clinically applicable model of sex steroid ablation, chemical castration using leuprolide acetate, and have shown enhanced T cell reconstitution following allogeneic BMT. We built on previous studies done by ourselves and others, studying the effects of sex steroid ablation on immune reconstitution (15–18, 24, 25), and demonstrated that chemical castration increases early hematopoietic progenitors in the BM as well as thymocyte subsets and splenic T cell numbers without exacerbating GVHD and maintaining GVT activity.

Previous studies have demonstrated that early hematopoietic progenitors are sex steroid sensitive. In this study, we also observed an increase, following chemical castration, in donor-derived LSK cells, CLPs, and myeloid progenitors following
FIGURE 6. T cell function is augmented in leuprolide acetate-treated mice at all time points after allogeneic BMT. Interestingly, we observed differences in the thymus earlier than those seen in the BM, suggesting a direct effect of sex steroid ablation on the thymus. Sex steroid receptors are expressed on all thymocyte subsets (30) as well as medullary and cortical thymic epithelial cells (31). LHRH receptors are expressed on cells in the thymus (12), although little is known about the cellular distribution. In this study, we used leuprolide acetate, an LHRH agonist, to chemically castrate BMT recipients; the leuprolide acetate may have direct effects on thymic reconstitution or indirect effects via the ablation of sex steroids.

Previous studies have documented that sex steroids effect both thymocytes and the thymic stroma. Surgical or chemical castration reverses and delays the onset of age-related thymic atrophy (8, 11, 32–40), while the administration of sex steroids reverses these effects and also exacerbates normal thymic atrophy (9, 41–46). There is evidence to suggest that sex steroid ablation independently affects both the thymic stroma and the earliest T cell progenitors. Olsen et al. (31) demonstrated that the presence of a functional androgen receptor on the thymic stroma is essential for androgen-dependent thymic atrophy to occur. Although Heng et al. (18) demonstrated that early T cell progenitor (ETP) numbers were increased as early as 5 days after castration of 9-mo-old mice (18), (12) although little is known about the cellular distribution. In this study, we observed differences in the thymus earlier than those seen in the BM, suggesting a direct effect of sex steroid ablation on the thymus. Sex steroid receptors are expressed on all thymocyte subsets (30) as well as medullary and cortical thymic epithelial cells (31). LHRH receptors are expressed on cells in the thymus (12), although little is known about the cellular distribution. In this study, we used leuprolide acetate, an LHRH agonist, to chemically castrate BMT recipients; the leuprolide acetate may have direct effects on thymic reconstitution or indirect effects via the ablation of sex steroids.

Although the increase in myeloid progenitor cells did not lead to an increase in the number of donor-derived myeloid cells in the bone marrow or the periphery, we did observe a significant increase in donor-derived B cell numbers in the BM. This is in agreement with previous studies that have demonstrated that sex steroids, both androgens and estrogens, have a suppressive effect on B lymphopoiesis, while their withdrawal enhances B cell development (7, 27–29).

Thymic cellularity was significantly increased in leuprolide acetate-treated mice at all time points after allogeneic BMT. Interestingly, we observed differences in the thymus earlier than those seen in the BM, suggesting a direct effect of sex steroid ablation on the thymus. Sex steroid receptors are expressed on all thymocyte subsets (30) as well as medullary and cortical thymic epithelial cells (31). LHRH receptors are expressed on cells in the thymus (12), although little is known about the cellular distribution. In this study, we used leuprolide acetate, an LHRH agonist, to chemically castrate BMT recipients; the leuprolide acetate may have direct effects on thymic reconstitution or indirect effects via the ablation of sex steroids.

FIGURE 6. T cell function is augmented in leuprolide acetate-treated mice while GVHD is not exacerbated and GVT activity is maintained. A–D, Eight- to 12 wk-old male BALB/c mice were lethally irradiated and transplanted with 5 × 106 B6.SJL (Ly5.1) TCD BM cells. Mice were infected with leuprolide acetate 28 days before BMT mice were injected with leuprolide acetate 28 days before BMT and proliferation was assessed. A, Anti-CD3/anti-CD28 double-plate-bound proliferation assay of spleen cells. n = 5 and ∗, p ≤ 0.05. B, Third-party MLR. Splenocytes were stimulated with TCD B10.BR splenocytes. C, Intracellular staining for IFN-γ. CD4 and CD8 T cells were stained for IFN-γ production following transplantation. D, T cell-dependent B cell response, mice were vaccinated with NP23-CGG, percentage and number of plasma cells and the number of Ag-specific plasma cells were determined by ELISPOT. E, GVHD. Eight- to 12 wk-old male BALB/c mice were lethally irradiated and transplanted with 5 × 106 B6.SJL (Ly5.1) TCD BM cells and 5 × 106 or 2 × 106 T cells. 28 days before BMT mice were injected with LHRH agonist, n = 10. F, GVMT. Eight- to 12-wk-old B6D2F1 mice were lethally irradiated and received 1 × 107 P815 mastocytoma cells and 5 × 106 T cells, both of C57BL/6 origin.

Moreover, we observed differences in the thymus earlier than those seen in the BM, suggesting a direct effect of sex steroid ablation on the thymus. Sex steroid receptors are expressed on all thymocyte subsets (30) as well as medullary and cortical thymic epithelial cells (31). LHRH receptors are expressed on cells in the thymus (12), although little is known about the cellular distribution. In this study, we used leuprolide acetate, an LHRH agonist, to chemically castrate BMT recipients; the leuprolide acetate may have direct effects on thymic reconstitution or indirect effects via the ablation of sex steroids.

Interestingly, in this study, we were unable to identify ETPs in either group up to 42 days after allogeneic BMT. This finding is in agreement with an earlier study which demonstrated that early thymic reconstitution following BMT occurs in the absence of ETPs (48). However, in this study, increases in TN subsets were observed as were increases in DP and mature SP thymocytes.

Although there has been much speculation as to the mechanism of sex steroid suppression of lymphopoiesis, there have been few chemical castration. Although little is known about the effects of androgens on BM progenitors, several studies have addressed the effects of estrogen. Estrogen can act both directly on BM progenitor cells and indirectly via the BM stroma (4, 26, 27), resulting in a decrease in lymphoid progenitor potential. This is thought to be due to a decrease in lymphoid-committed precursors in the Lin−c-kihih population, as well as a depletion of Lin−IL-7Rα−c-kihih population of cells that contain CLPs (4). LSK cells are significantly decreased following 17β-estradiol (E2) treatment of ERα-replete mice (26). Interestingly, more modest decreases are also seen in ERα knockout mice, suggesting an ERα-independent component. BM chimeras of ERα-replete and knockout mice showed that the presence of ERα on the hematopoietic cells was essential for the dramatic loss of LSK cells (26).
TGF-β production is decreased in the thymus and BM following androgen treatment, making this suppressive growth factor a major candidate for sex steroid-induced suppression of lymphopoiesis (49, 50). In aged mice, surgical castration did not appear to alter the mRNA expression of TGF-β1, IL-7, or keratinocyte growth factor (KGF) (24). While following autologous BMT, we found that TGF-β1 was decreased in castrated mice in both thymic stromal cells and BM cells, while thymic stromal cell production of IL-6 was also decreased in castrated mice (15). Castrated allogeneic BMT recipients had decreased TGF-β1 and increased production of KGF in the BM, while no obvious changes were observed in the thymus (16).

One of the most well-studied growth factors in the aging setting is the cytokine IL-7. The majority of studies find a decrease in thymic IL-7 production with age (51–53), but treatment of very old mice with IL-7 does not result in increased thymic output (54). Although intrathymic injection of IL-7-producing stromal cells enhances early thymocyte development (55) and IL-7 treatment of old mice can reverse age-related increases in thymic apoptosis and enhance early T cell development (51), IL-7 treatment does not reverse age-related involution (55). Together, these findings suggest that a decline in IL-7 production with age may play a part in the dysfunction of age-related T cell development, but may not be involved in the involution process. IL-7 production is severely affected for an extended period following irradiation (56), suggesting that it may not play a role in castration-enhanced immune reconstitution following HSC transplantation.

T cell reconstitution, both thymic and peripheral, was enhanced after syngeneic and allogeneic BMT and KGF treatment (57). The same study showed that the thymi of KGF-treated HSC transplanted mice contained more IL-7 mRNA+ cells, suggesting a role for IL-7 in KGF-mediated enhanced immune reconstitution (57). KGF treatment is also known to facilitate engraftment and enhance BM reconstitution following allogeneic BMT (58). Therefore, the increase in KGF expression we observed in an earlier study (16) may play a role in the enhanced immune reconstitution observed following allogeneic BMT and sex steroid ablation.

In this study, we have shown that the increased thymic reconstitution observed after allogeneic BMT and chemical castration leads to an increase in donor-derived splenic CD4+ and CD8+ T cells. The significant increase was observed only at the late time point of 42 days after BMT. This is not surprising, considering it takes as long as 28 days for a cell to mature from BM precursor to mature T cell (59, 60). The most striking increase was observed in the naive compartment of both CD4+ and CD8+ cells, suggesting an increase in thymic T cell production and export. Interestingly, there was an increase in memory populations as well. This may be due to the direct effects of leuprolide acetate on peripheral T cells that express LHRH receptors.

Using in vitro (proliferation assays and cytokine production) and in vivo (T cell-dependent B cell response to NP23-CGG) assays, we have demonstrated that the enhanced donor-derived T cell reconstitution observed following allogeneic BMT and leuprolide acetate treatment translates into a significant increase in T cell function.

Enhanced peripheral T cell function is an advantageous outcome in most settings. However, following allogeneic BMT, the exacerbation of GVHD as a consequence of therapies designed to enhance immune reconstitution is a grave concern. In this study, castration did not worsen GVHD and GVTT activity remained intact, further supporting its potential use as an adjunct therapy following treatments that result in immunosuppression results.

The data presented in this study add significantly to a recent nonrandomized clinical study that compared leuprolide acetate-treated allogeneic and autologous BMT recipients to age-matched controls (61). We have demonstrated, in a clinically relevant model of allogeneic BMT, that the enhanced peripheral T cell reconstitution is due to both increases in lymphoid-committed precursors as well as enhanced thymic regeneration. Furthermore, GVHD was not exacerbated and GVTT activity was maintained. These data suggest that leuprolide acetate treatment may be a novel strategy to enhance posttransplant T cell reconstitution.

Disclosures
The authors have no financial conflict of interest.

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