IL-7 mediated protection against minor antigen-mismatched allograft rejection is associated with enhanced recovery of regulatory T cells

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ABSTRACT

Background and Objectives

Interleukin-7 (IL-7) has been studied for its possible immunorestorative capacities following stem cell transplantation and has been shown to enhance post-transplant immune recovery predominantly by peripheral T-cell expansion. A major concern of IL-7 is its possible aggravating effect on graft-versus-host and host-versus-graft reactivity.

Design and Methods

To study the effect of IL-7 on host-versus-graft reactivity, we applied IL-7 in an experimental transplantation model using RAG-1$^{-/-}$ mice supplied with B6 CD45.1 congenic T cells as recipients of T-cell depleted allogeneic bone marrow grafts.

Results

Rejection of minor antigen-mismatched bone marrow was significantly reduced in IL-7 treated recipients compared with PBS treated control mice. Rejection was observed in 2 out of 18 IL-7 treated mice compared with 9 out of 17 PBS treated mice (11% vs. 53%; p=0.012). IL-7 administration resulted in enhanced recovery of peripheral blood CD4$^+$CD25$^+$ regulatory T cells (Treg) with a concomitant increase in peripheral blood Foxp3 mRNA expression. IL-7Rα (CD127) was expressed by the vast majority of CD4$^+$Foxp3$^+$ T cells. The incidence of graft rejection following fully MHC mismatched bone marrow transplantation was not reduced nor enhanced by IL-7 administration.

Interpretation and Conclusions

Post-transplant IL-7 administration protects against minor antigen-mismatched bone marrow rejection, which may be due to enhanced Treg recovery.

Key words: interleukin-7, regulatory T cells, graft rejection, bone marrow transplantation.

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The histocompatibility barrier between donor and recipient manifested as either graft-versus-host (GVH) or host-versus-graft (HVG) reactions continue to affect outcome after allogeneic hematopoietic stem cell transplantation (allo-SCT).[1] Graft-versus-host disease (GVHD), mediated by immunocompetent donor-derived T cells recognizing genetically disparate host cells, is one of the major causes of death following allo-SCT.[2] While advances in recipient-donor matching by molecular HLA typing and improvement of pre-transplant conditioning measures have reduced graft rejection, allo-SCT from alternative donors and allo-SCT following reduced intensity conditioning may still be complicated by impaired engraftment and overt graft rejection.[3-6] Apart from immunosuppressive agents administered prior to transplantation, the intensity of postgrafting immunosuppression has been shown to affect HVG reactivity and the incidence of rejection.[7-9] Data collected from various murine models demonstrate that $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) are important mediators of postgrafting immunosuppression. Pre-transplant depletion of recipient $CD25^+$ T cells has been shown to reduce levels of donor engraftment and the adoptive transfer of large numbers of $CD4^+CD25^+$ Treg at the time of allo-SCT may prevent bone marrow graft rejection.[10-12] The lymphopoietic cytokine interleukin-7 (IL-7) has been studied for its possible immunorestorative capacities following SCT in murine and non-human primate models. Whereas IL-7 administration might affect thymopoiesis, IL-7 is most importantly identified as key regulator of homeostatic peripheral T-cell expansion (HPE).[13-22] A major concern of post-transplant administration of IL-7 is its possible aggravating effect on GVH and HVG reactivity. Reports concerning the effect of IL-7 on the incidence and severity of GVH have given conflicting results.[15,23,24] Until now, there have been no studies reporting the effect of IL-7 on HVG alloreactivity. Therefore, we applied IL-7 in experimental murine stem cell transplantation models with major and minor histocompatibility barriers. We show that IL-7 administration protects against minor antigen-mismatched allograft rejection, which is associated with an increased recovery of Treg.

Design and Methods

Mice

C57BL/6-RAG-$1^{-/-}$ mice, originally obtained from The Jackson Immunoresearch Laboratories (Bar Harbor, ME), and C57BL/6-Ly5.1 mice were bred at the Experimental Animal Centre, Erasmus University Medical Center, Rotterdam, the Netherlands. 129Sv and Balb/C mice were purchased from Charles River Laboratories (Wilmington, MA, USA). Mice were maintained under specific pathogen–free conditions in individual ventilated cages with acidified water and antibiotics. All animal procedures were performed in accordance with protocols approved by the local Committee for Animal Experiments.

Bone marrow transplantation

Bone marrow obtained from crushed femurs and tibias of donor mice was depleted of T cells by incubation with rat antimouse CD4 (YTS191, YTA312) and rat antimouse CD8 (YTS169) monoclonal antibodies (mAbs)[25] followed by magnetic separation using the AutoMacs according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The efficacy of T-cell depletion was monitored by flow cytometry and was always found to be more than 2 log. Splenic T cells were obtained by negative selection using a cocktail of non–T-cell monoclonal antibodies according to the manufacturer's instructions (StemSep; Stem cell Technologies, Vancouver, BC, Canada). Purity of the T-cell fraction was always found to exceed 95% as confirmed by flow cytometry. Ten- to 14-week-old RAG-$1^{-/-}$ mice supplied with graded doses of purified $CD45.1^+$ congenic C57BL/6 T cells received either 12.5×10^6 T-cell depleted 129Sv bone marrow cells (minor antigen-mismatched) or 6×10^6 T-cell depleted Balb/C bone marrow cells (fully MHC mismatched) by tail vein infusion (0.5 mL total volume) preceded by 3 and 6 Gy of total body irradiation respectively (^{137}Csγ-source, Gammacell, Atomic Energy of Canada, Ottawa, Canada). During the 6-7 weeks following transplantation, mice received a daily subcutaneous injection of either PBS or 1 μg of recombinant human IL-7 (0.25 mL total volume), which was kindly provided by Dr Michel Morre (Cytheris, Vanves, France). Rejection was defined as a sustained peripheral blood $CD45.2^+CD3^+$ T-cell number below 50 cells/μL, which was monitored by flow cytometry at weekly intervals.

Flow cytometric analysis

At serial time points following transplantation, blood was collected from the murine retro-orbital plexus. Absolute numbers of peripheral blood lymphocytes were determined by a single-platform flow cytometric assay as previously described.[18] MAbs used for flow cytometric analysis were fluorescein isothiocyanate (FITC)–conjugated anti-CD3, and anti-CD45.1 (Becton Dickinson, San Jose, CA, USA); phycoerythrin (PE)–conjugated anti-CD19, anti-CD45.1, anti-CD4 (Becton Dickinson), anti-CD127 (e-Bioscience), anti-CD8 (Beckman Coulter), and anti-IgG2a (BD Pharmingen, Alphen a/d Rijn, the Netherlands); Cy-Chrome–conjugated anti-CD45; allophycocyanin (APC)–conjugated anti-CD4, and anti-CD25 (Becton Dickinson); biotin-conjugated anti-CD45.2 (Becton Dickinson). Streptavidin-PE and streptavidin-APC (Becton Dickinson) were used to detect biotinylated monoclonal antibodies. The expression of Foxp3 was analysed by intracellular staining with anti-Foxp3 (e-Bioscience) after fixation and permeabilization of peripheral blood cells according to the manufacturer's instructions (e-Bioscience, San Diego, CA, USA). Intracellular IL-4 and IFN-γ staining was performed on single cell suspensions prepared from spleen. Two million spleen cells were stimulated with either 25 ng/mL of phorbol-12-myristate 13-

acetate (PMA; Sigma) and 1 μg/mL of ionomycin (Sigma) or with medium for 5 hours at 37°C and 5% CO2. Brefeldin A (Sigma) was added at a final concentration of 10 μg/mL for the final 4 hours of incubation. Cells were then stained with anti-CD45.1 and anti-CD4 monoclonal antibodies and after fixation and permeabilization (BD Pharmingen), cells were stained with anti-IL-4 and anti-IFN-γ (BD Pharmingen). Activation of the cells was confirmed by analysis of CD69 expression, which was over 95% in all stimulated samples. All analyses were performed in duplicate. All flow cytometric analyses were performed using a FACSCalibur (Becton Dickinson). Flow cytometric data were collected and analyzed using CELLQuest software (Becton Dickinson).

In vitro Treg activity assay

$CD4^+CD25^{high}$ Treg were isolated from spleen cells of IL-7 treated mice using a FACSAria cell sorter (Becton-Dickinson). The purity of the sorted cell population was always found to exceed 95% as confirmed by flow cytometry. Sorted $CD4^+CD25^{high}$ Treg cells from IL-7-treated mice (5×10^4) were evaluated for their ability to suppress T-cell proliferation by co-culture with sorted $CD4^+CD25^-$ C57BL/6 responder T cells (5×10^4) that were stimulated with 0.5 μg/mL anti-CD3 antibody and RAG-$1^{-/-}$ spleen cells (2×10^5) as antigen presenting cells. Cultures were performed in 96-well U-bottom plates. Responder cells with or without Treg were cultured in RPMI 1640 medium at 37°C and 5% CO2 for 3 days. Tritium thymidine (^{3}H-TdR) was added at 1 μCi/well for the last 18 hours of culture. All assays were performed in quadruplicate.

Real-time quantitative PCR of Foxp3

RNA was purified from blood using the Qiagen Blood Mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions after which cDNA was synthesized. The PCR was performed in a 25-μL reaction containing 20 μL of PCR mix Sybergreen (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands), 20 pmol/mL forward- and 20 pmol/mL reverse primer (Invitrogen, Merelbeke, Belgium), 6 μL sterile water) and 5 μL of cDNA. All reactions were performed in duplicate. PCR conditions were 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Sequences of the Foxp3 primers were: forward primer 5'-GCAATAGTTCCTTCCCAGAGTTCT-3' and reverse primer 5'-GGATGGCCCATCGGATAAG-3'. To compensate for variations in input cDNA the constant gene segment of the ribonuclease inhibitor (RI) gene was used as endogenous reference gene. Sequences of the RI primers were: forward primer 5'-TCCAGTGTGAGCAGCTGAG-3' and reverse primer 5'-TGCAGGCACTGAAGCACCA-3'. Foxp3 mRNA was detected with real-time quantitative RT-PCR using the ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, CA, USA) and computer software package SDS2.2. The mean threshold cycles (Ct) of the duplicates were used to calculate the expression of Foxp3 mRNA relative to the RI-control. Relative Foxp3 transcripts were calculated by the ΔCt method: $\Delta Ct = \Delta Ct_{Foxp3} - \Delta Ct_{RI}$. Relative Foxp3 transcript = $1/2^{\Delta Ct}$.

Statistical analysis

The Mann-Whitney-U test was used to compare numbers of peripheral blood $CD3^+$ T-cell subsets and peripheral blood Foxp3 expression between PBS- and IL-7 treated recipient mice and to compare Foxp3 expression in mice with and without graft rejection. Fisher's exact test was used to compare the incidence of graft rejections between PBS- and IL-7 treated transplant recipients. All reported P-values are 2-sided, and a significance level of $\alpha \leq 0.05$ was used.

Results

IL-7 and allograft rejection

Peripheral homeostatic expansion of T cells has been shown to be the pre-dominant effect of exogenous IL-7 after T-cell replete BMT.[18] Given this, we decided to evaluate the effect of IL-7 administration on host-versus-graft alloreactivity. Three Gy irradiated C57BL/6-RAG-$1^{-/-}$ mice, supplied with escalating numbers of B6 CD45.1 congenic T cells, received an allogeneic MHC-matched minor antigen-mismatched T-cell depleted 129Sv bone marrow graft followed by IL-7 or PBS administration from day 1 until day 42. Engraftment was monitored at weekly intervals. The incidence of minor antigen-mismatched graft rejection depended on the numbers of B6 CD45.1 T cells supplied prior to transplantation (Figure 1). Post-transplant administration of IL-7 reduced the incidence of graft rejections in mice supplied with 10^5 B6 T cells. Rejection was observed in 2 out of 18 IL-7 treated mice compared with 9 out of 17 PBS treated mice (11% vs. 53%; p=0.012). No rejections were observed in recipients of 1×10^4 B6 T cells and recipients of 1×10^6 B6 T cells all rejected their minor antigen-mismatched bone marrow graft irrespective of IL-7 treatment. Results are shown in Table 1. As shown before,[18] IL-7 administration resulted in a sustained increase in numbers of B6 $CD45.1^+$ T cells compared with PBS. At day 42 after BMT, PBS treated mice supplied with 1×10^5 B6 T cells showed a mean number of 217 cells/μL (range: 41-707) compared with 578 cells/μL (range: 54-1,344) in IL-7 treated mice (p=0.02; Figure 2A). Thus, post-transplant IL-7 administration reduced the incidence of graft rejection despite an increase in the numbers of peripheral blood congenic B6 T cells.

Like the analysis of IL-7 and minor antigen-mismatched bone marrow rejection, experiments were also performed using 6 Gy irradiated C57BL/6-RAG-$1^{-/-}$ mice supplied with escalating numbers of B6 CD45.1 congenic T cells as recipients of 6×10^6 T-cell depleted Balb/C bone marrow to study the effect of IL-7 on MHC mismatched graft rejection. No difference in the frequency of graft rejection was observed between PBS and IL-7 treated recipients. In PBS

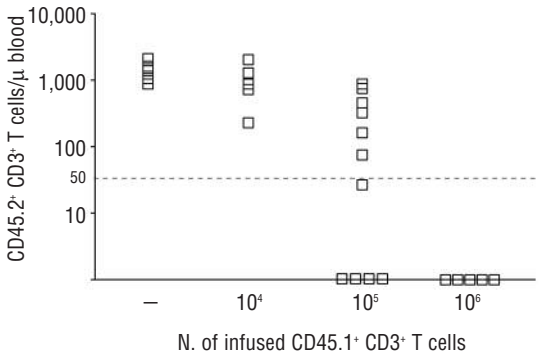

Figure 1. Allograft rejection following minor antigen-mismatched BMT. 3 Gy irradiated C57BL/6-RAG-1$^{-/-}$ supplied with escalating numbers of congenic B6 T cells received 12.5×10^6 MHC matched minor antigen-mismatched T-cell depleted 129Sv bone marrow cells. Bone marrow derived 129Sv T cells and supplied B6 T cells were distinguished by CD45.2 and CD45.1 expression respectively. Rejection was defined as a sustained peripheral blood CD45.2$^+$CD3$^+$ T-cell number below 50 cells/µL. Bone marrow derived CD45.2$^+$CD3$^+$ T cell numbers at day 42 are shown for all individual recipients of no T cells (n=5), 10^4 T cells (n=5), 10^5 T cells (n=11) and 10^6 T cells (n=5).

Table 1. Incidence of graft rejection following minor antigen-mismatched BMT.

	Numbers of infused B6 T cells			
	0	*10⁴*	*10⁵*	*10⁶*
Treatment modality				
PBS	0/5*	0/5	9/17	5/5
IL-7	nd†	0/5	2/18	5/5

**Number of allograft rejections per total number of performed transplantations.*
†Not determined.

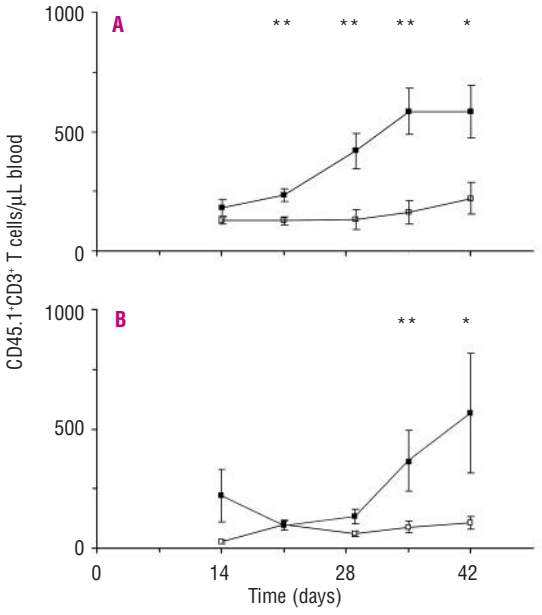

Figure 2. IL-7 mediated peripheral T-cell expansion. C57BL/6-RAG-1$^{-/-}$ supplied with escalating numbers of congenic B6 CD45.1$^+$ T cells received either 3 Gy irradiation followed by a T-cell depleted minor antigen-mismatched BMT or 6 Gy irradiation followed by a fully MHC mismatched BMT. PBS or IL-7 were administered subcutaneously (1 µg daily) from day 1 to day 42. Peripheral expansion of B6 T cells was studied by single-platform flow cytometry of peripheral blood samples taken at weekly intervals. Mean absolute numbers (± SEM) of CD45.1$^+$CD3$^+$ T-cells/µL blood are shown for (A) PBS (⅂, n=14) and IL-7 (L, n=16) treated recipients of 10^5 B6 T cells and a minor antigen-mismatched BM graft (129Sv) and (B) PBS (⅂, n=8) and IL-7 (L, n=9) treated recipients of 10^3 B6 T cells and a MHC mismatched BMT (Balb/C). *p<0.05 and **p<0.01.

treated mice supplied with 1×10^3 B6 T cells, 4 out of 8 transplantations ended in rejection compared to 5 out of 9 transplantations in IL-7 treated mice (50% vs. 56%). All recipients of 1×10^4 and 1×10^5 B6 T cells rejected their MHC-mismatched allograft. Comparable to minor antigen-mismatched BMT, administration of IL-7 following fully MHC mismatched transplantation resulted in expansion of the supplied B6 CD45.1 congenic T cells. IL-7 treated mice supplied with 1×10^3 B6 T cells had a mean number of 566 cells/µL (range: 32-2,512) at day 42 as compared to 107 cells/µL (range: 14-296) in PBS treated mice (p=0.04; Figure 2B).

IL-7 enhances BM- and congenic T-cell derived Treg recovery following MHC-matched minor antigen-mismatched BMT

IL-7 administration following minor antigen-mismatched allo-SCT resulted in fewer rejections despite peripheral expansion of mature congenic T cells. As Treg are known to be important mediators of postgrafting immunosuppression, we evaluated the effect of IL-7 treatment on endogenous Treg recovery by flow cytometric analysis of cell surface co-expression of CD4 and CD25. Absolute numbers of BM- and congenic T-cell derived CD4$^+$CD25^{high} T cells were determined in peripheral blood samples taken at weekly intervals after BMT. Mice treated with IL-7 showed an increased recovery of both BM derived CD4$^+$ and CD4$^+$CD25^{high} T cells compared to PBS treated mice (Figure 3). The frequency of CD4$^+$CD25^{high} Treg within the CD45.2$^+$CD4$^+$ T-cell pool was similar in IL-7 treated and PBS treated mice. Next we studied the effect of IL-7 administration on the CD4$^+$CD25^{high} Treg present in the supplemented congenic CD45.1 T-cell pool. IL-7 treatment not only resulted in increased numbers of CD45.1$^+$CD4$^+$ T cells, but also in a moderate increase in numbers of CD45.1$^+$CD4$^+$CD25^{high} Treg (Figure 3). Then we analysed the suppressive ability of CD4$^+$CD25^{high} T cells from IL-7 treated mice. Sorted CD4$^+$CD25^{high} T cells, selected from spleens of IL-7 treated mice 35 days after transplantation, inhibited the anti-CD3-induced proliferation of CD4$^+$CD25$^-$ T cells *in vitro,* demonstrating their regulatory capacity (Figure 4).

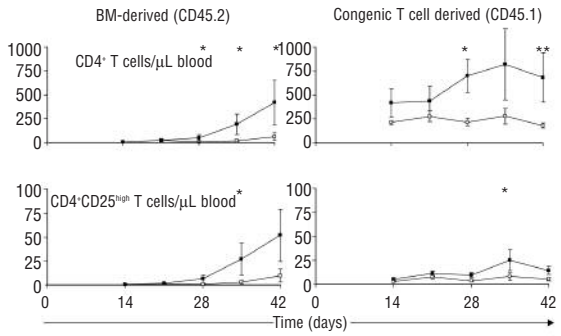

Figure 3. IL-7 enhances BM- and congenic T-cell derived $CD4^+$ and $CD4^+CD25^{high}$ T-cell recovery. 3 Gy irradiated C57BL/6-RAG-$1^{-/-}$ supplied with 10^5 congenic B6 $CD45.1^+$ T cells received 12.5×10^6 T-cell depleted 129Sv bone marrow cells. PBS or IL-7 were administered subcutaneously (1 µg daily) from day 1 until day 49. Peripheral blood $CD4^+$ and $CD4^+CD25^{high}$ T-cell recovery were studied by single platform flow cytometry. Mean absolute numbers (± SEM) of BM derived $CD4^+$ and $CD4^+CD25^{high}$ T cells/µL blood and congenic T-cell derived $CD4^+$ and $CD4^+CD25^{high}$ T cells/µL blood in PBS treated mice (l, n=6) and IL-7-treated mice (L, n=6) are shown. *p<0.05 and **p<0.01.

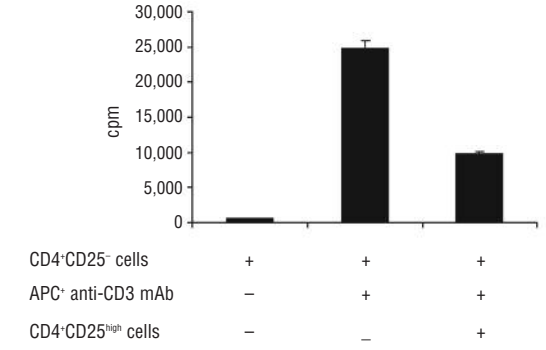

Figure 4. $CD4^+CD25^{high}$ Treg from IL-7-treated mice have suppressive ability *in vitro*. Sorted $CD4^+CD25^-$ T cells from C57BL/6 mice (5×10^4) stimulated with anti-CD3 mAb (0.5 µg/mL) and RAG-$1^{-/-}$ spleen cells (2×10^5) as antigen presenting cells (APC) were cultured in the presence or absence of sorted $CD4^+CD25^{high}$ Treg from IL-7-treated mice (5×10^4). T-cell proliferation was measured by ^{3}H-TdR incorporation as shown by mean counts per minute (cpm ± SD) of quadruplicate samples.

IL-7 enhances Foxp3 expression in peripheral blood cells of Rag-$1^{-/-}$ mice following MHC matched minor-antigen mismatched BMT

Since Foxp3 is a more selective marker for murine Treg, Foxp3 expression was determined by real-time quantitative RT-PCR of Foxp3 mRNA expression in peripheral blood at day 21 and day 49 after BMT. At day 21, the expression of Foxp3 mRNA in the IL-7 treated mice was higher than in PBS treated mice. PBS treated recipients showed a mean expression of 165×10^{-4} (range: 42-313) relative transcripts compared with a mean expression of 436×10^{-4} (range: 146-947) relative transcripts in IL-7 treated mice (p=0.04; Figure 5).

At day 49, IL-7 treated mice showed a strong increase in expression of Foxp3 mRNA compared with the expression at day 21, whereas Foxp3 mRNA expression in PBS treated mice was only moderately enhanced. In PBS treated mice, a mean expression of 267×10^{-4} (range: 17-994) relative transcripts was detected compared with a mean expression of 1150×10^{-4} (range: 167-2,320) relative transcripts in IL-7 treated mice (p=0.02; Figure 5). Furthermore, Foxp3 mRNA expression levels inversely correlated with rejections irrespective of IL-7 treatment. Mice that did not reject their bone marrow graft had a higher mean expression of Foxp3 mRNA compared with mice that rejected their graft. In the no-rejection group, mice had a mean Foxp3 expression of 1316×10^{-4} (range: 994-2,320) relative transcripts whereas mice that rejected their graft had a mean Foxp3 expression of 129×10^{-4} (range: 17-193) relative transcripts (p=0.006; Figure 5). As our data indicate that $CD4^+Foxp3^+$ Treg respond to IL-7, we analyzed CD127 (IL-7Rα) expression on $CD3^+CD4^+Foxp3^+$ Treg of normal B6 mice by four-color flowcytometry. $CD3^+CD4^+Foxp3^+$ Treg did indeed express CD127 although at a lower level of expression than $CD3^+CD4^+Foxp3^-$ T cells. This is in line with recently published observations by Liu *et al.* showing a lower CD127 expression on murine $CD4^+Foxp3^+$ T cells compared with $Foxp3^-$ T cells.[26] Results are shown in Figure 6.

IL-7 treatment does not alter the Th1/Th2 balance in RAG-$1^{-/-}$ recipients of MHC matched minor-antigen mismatched BMT

Since Th2 and Tc2 populations play an important role in the prevention of GVH- and HVG reactions,[27] the

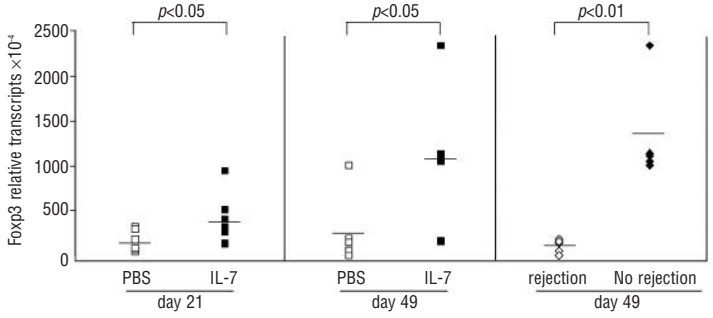

Figure 5. IL-7 enhances Foxp3 expression in peripheral blood cells. 3 Gy irradiated C57BL/6-RAG-$1^{-/-}$ supplied with 10^5 congenic B6 $CD45.1^+$ T cells received 12.5×10^6 T-cell depleted 129Sv bone marrow cells. PBS or IL-7 were administered subcutaneously (1 µg daily) from day 1 until day 49. Foxp3 mRNA and RI mRNA were determined by real-time quantitative RT-PCR of peripheral blood samples taken at day 21 and day 49 after BMT. Relative Foxp3 expression is shown for PBS (l) and IL-7 (L) treated mice at day 21, PBS (l) and IL-7 (L) treated mice at day 49 and mice with (r) and without (R) graft rejection irrespective of cytokine treatment (n=5-6 mice per group).

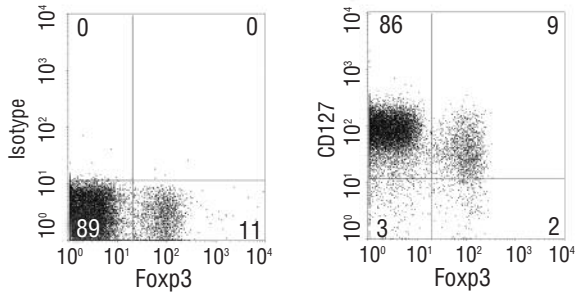

Figure 6. CD4⁺Foxp3⁺ regulatory T cells express CD127. Peripheral blood cells from normal B6 mice were stained with CD3, CD4, Foxp3 and IgG2a or CD127 mAb and analyzed by four-color flow cytometry. Cells were gated on CD3⁺CD4⁺ lymphocytes and analysed for CD127 and Foxp3 expression. Dotplots of isotype- and CD127- stained CD3⁺CD4⁺ T cells as representative examples of 7 individual mice are shown.

observed protective effect of IL-7 on host-versus-graft reactivity following MHC matched minor-antigen mismatched BMT might well be a direct effect on T-cell phenotype and cytokine profile thus altering the Th1/Th2 balance. Therefore, we evaluated the effect of IL-7 on the frequency of IFN-γ producing Th1 cells and IL-4 producing Th2 cells. RAG-1$^{-/-}$ recipients of MHC matched minor-antigen mismatched BMT were sacrificed at day 35 post-transplant and spleens were harvested. Cells were stimulated with PMA and ionomycin to increase cytokine production as no cytokine signals could be detected without stimulation. The percentage of splenic IFN-γ-producing T cells within the CD45.1⁺CD4⁺ T-cell compartment was approximately 60-70% both in PBS and IL-7 treated mice. Furthermore, percentages were similar in mice that did and mice that did not reject the bone marrow graft. Results are shown in Figure 7. Since numbers of IL-4 producing CD45.1⁺CD4⁺ T cells were always below 5% in both groups of mice, irrespective of rejection (*data not shown*), the Th1/Th2 balance was unaffected by IL-7 treatment.

Discussion

IL-7 has been studied for its possible immunorestorative capacities following SCT and has been shown to enhance post-transplant immune recovery predominantly by peripheral T-cell expansion.[13-22] A major concern of post-transplant administration of IL-7 may be its possible aggravating effect on GVH and HVG reactivity by enhancement of the allo-antigen-reactive T-cell population. Reports of the effect of IL-7 on GVH reactions have yielded conflicting results and at present it is unknown whether IL-7 may affect HVG alloreactivity.[15,23,24] Therefore, we applied IL-7 in an experimental stem cell transplantation model using RAG-1$^{-/-}$ mice supplied with B6 CD45.1 congenic T cells to study the effect of IL-7 administration on HVG reactivity following SCT across major and minor histocompatibility barriers. We show that post-transplant administration of IL-7 results in a reduced incidence of allograft rejection in recipients of minor antigen-mismatched bone marrow.

Several possible explanations for the observed protective effect were considered. As postgrafting immunosuppression is important for prevention of graft rejection[7,8] and CD4⁺CD25⁺Foxp3⁺ Treg are important mediators of postgrafting immunosuppression,[28] we first hypothesized that IL-7 might reduce allograft rejection by increasing the number of Treg after BMT. In our longitudinal analysis of T-cell recovery in recipients of MHC matched minor antigen-mismatched bone marrow cells, we did indeed find an enhanced recovery of CD4⁺CD25^{high} T cells and increased levels of Foxp3 mRNA in IL-7 treated mice compared with PBS treated-control mice. Furthermore, significantly higher levels of Foxp3 mRNA were measured in all mice that did not reject the marrow graft compared with mice that did reject the graft. Early after transplantation, all Treg are derived from the supplied CD45.1 congenic mature T cells. From day 21 onwards, Treg originating from the bone marrow appear and gradually become the major population of peripheral blood Treg. Both populations of Treg are increased by IL-7 treatment and may have contributed to the observed reduction in HVG reactivity. Our findings compare well to several other reports showing that adoptive transfer of Treg may prevent bone marrow graft rejection.[10,12,29] Rejection of MHC-matched minor antigen-mismatched bone marrow is a lengthy process that may be modulated both early and later after transplantation. Early after bone marrow transplantation, adoptively transferred CD62L^{hi} Treg suppress bone marrow

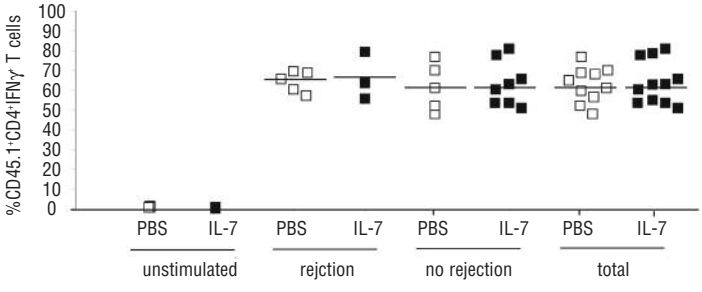

Figure 7. IL-7 treatment does not alter the Th1/Th2 balance in RAG-1$^{-/-}$ recipients of MHC matched minor-antigen mismatched BMT 3. Gy irradiated C57BL/6-RAG-1$^{-/-}$ supplied with 10^5 congenic B6 CD45.1⁺ T cells received 12.5×10^6 T-cell depleted 129Sv bone marrow cells. Flow cytometric analysis of splenic IFN-γ producing congenic T-cell derived CD45.1⁺CD4⁺ T cells was performed at day 35 post-transplant both in PBS (▫) and IL-7 (▪) treated recipient mice either with or without graft rejection. The percentage of CD45.1⁺CD4⁺IFN-γ⁺ T cells in PBS and IL-7 treated mice without stimulation and after stimulation with PMA and ionomycin is shown.

rejection by suppression of the priming of alloreactive T cells in secondary lymphoid organs.[10] At later stages, effector/memory like CD62E/P$^+$CCR5$^+$ Treg may migrate into peripheral sites to suppress the expansion, cytokine secretion and/or cytolytic function of alloreactive effector T cells.[30-32]

An alternative explanation for the observed protective effect of IL-7 could have been a direct immunosuppressive effect of IL-7 on T cells by affecting the Th1/Th2 balance. In our model of MHC matched minor-antigen mismatched BMT the Th1/Th2 balance was unaffected by IL-7 administration. Our observations are in line with reports by Alpdogan *et al*. showing that there is no significant difference in IFN-γ and IL-4 production between IL-7 and PBS treated transplant recipient mice.[15,20]

In contrast to minor antigen-mismatched bone marrow graft rejection, the incidence of fully MHC mismatched graft rejection was not affected by IL-7. Since the frequency of allo-MHC reactive T lymphocytes is much higher compared with the frequency of minor antigen reactive T lymphocytes, rejection following fully MHC mismatched BMT may be more difficult to suppress by Treg. Furthermore, natural killer (NK) cells, next to T lymphocytes, might have contributed to MHC mismatched graft rejection. NK cells have proven to be a barrier to engraftment of fully MHC mismatched bone marrow and although Treg are capable of inhibiting NK-cell function *in vitro*, the inhibitory effect might be overcome in conditions resulting in significant cytokine production such as acute infection or allo-SCT.[33,34]

IL-7 and its role in alloreactivity have been the subject of study in several reports which have so far all concerned GVH reactivity. Sinha and co-workers applied IL-7 in a MHC mismatched parent → F1 model and showed significantly increased GVH reactivity in IL-7 treated recipients of escalating *subthreshold* numbers of T cells.[23] By contrast, Alpdogan and co-workers showed significantly reduced GVH reactivity following MHC mismatched BMT (parent → F1) and no difference in GVH reactivity between PBS and IL-7 treated recipients of minor antigen-mismatched BMT.[15] Differences in the dose of IL-7 used and the duration of therapy might have contributed to the observed differences between the two studies. Recently, Gendelman *et al*. reported a higher incidence of GVH reactivity in IL-7 treated recipients of MHC mismatched bone marrow. However, this study was restricted to unirradiated mice. In irradiated transplant recipients, GVH reactivity was not aggravated by IL-7 treatment.[24] Collectively, these studies do suggest that IL-7 might significantly worsen GVH reactivity. By contrast, in this first study concerning the effect of IL-7 on HVG reactivity, alloreactivity was definitely not increased by IL-7. More importantly, we even observed diminished HVG reactivity following minor antigen-mismatched BMT, which was associated with enhanced recovery of Treg. So apart from its immunorestorative capacities, IL-7 might also be useful in inducing transplantation tolerance especially in the setting of T-cell depleted allogeneic stem cell grafts.

Authors' Contributions

AECB: designed and performed research, collected and analyzed data and wrote the paper; MB: designed and performed research, collected and analyzed data and wrote the paper; SJP: performed research, collected and analyzed data; E-JW: performed research; HS: designed research and critically revised the manuscript; BL: designed research and critically revised the manuscript; EB: designed research and wrote the paper; JJC: designed research and wrote the paper.

Conflict of Interest

The authors reported no potential conflicts of interest.

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