granules containing perforin. Products treated with LLME demonstrated a median depletion of 92% of CD56+ cells, 67 % of CD8+ cells, but only 37% of CD4+ cells. Patients initially received 10e4 (haploidentical donor, n = 3), 10e5 (HLA identical unrelated donor, n = 1), 10e6 (HLA identical sibling donor, n = 4), or 10e7 (HLA identical sibling donor, n = 1) CD3+ cells/kg in cohorts of 3, and were eligible for additional infusions at a one log higher dose after 28 days if there was no evidence of GVHD or the CD4 count was less than the target of 200 cells/μl. The initial dose was escalated by one log in subsequent cohorts if no complications were observed in the previous cohort. All patients had pre-DLI CD4 counts of <5.0 cells/μl, and received 1 to 3 infusions post PCT. Since ATG was used during PCT cytoablation, the first DLI infusions were administered after ATG levels were <2 mcg/ml. After DLI #1, 4 of 5 matched sibling recipients and 1 of 1 unrelated donor recipients demonstrated increased CD4+ counts with a median increase of 68 donor derived CD4+ cells/μl (range 57-460). Three of these patients achieved a CD4+ count above 200 after 1-3 total infusions. In contrast, none of the haploidentical recipients (receiving the smallest doses) had an increase in their CD4+ counts. Two patients developed mild GVHD (1 haploidentical Grade II, 1 matched sibling Grade I). In recovering patients, spectratype analysis of CD4+ cells comparing donor lymphocytes before LLME treatment with lymphocytes recovered from the patients post DLI demonstrated that 94% of the resolvable Vß families were equally complex. Perforin positive cells, as well as adenovirus and CMV specific lymphocytes, also recovered post PCT in these patients. These preliminary results suggest that LLME treated DLI can accelerate CD4+ reconstitution without causing severe GVHD.

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NON-GVHD-INDUCING CD62L$^-$ MEMORY T CELLS PROMOTE NEW T CELL REGENERATION FROM HEMATOPOIETIC STEM CELLS

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We previously demonstrated that allogeneic CD62L$^-$ memory T cells from unprimed donor mice are unable to induce graft-versus-host disease (GVHD) in allogeneic host (Blood. Prepublished October 9, 2003; DOI 10.1182/blood-2003-08-2987). In this published study, we also demonstrated that in contrast to bulk T cells and CD62L$^+$ T cells, CD62L$^-$ T cells contribute to post-transplant T cell reconstitution not only by peripheral expansion but also by promoting new T cells from T cell-depleted bone marrow. To investigate whether the new T cells were indeed generated from hematopoietic stem cells, we transplanted 1x10^6 CD62L$^-$ splenic T cells from C57BL/6 mice (H2^b, CD45.2) along with 5000 purified c-Kit$^+$Thy1.1^{low}Lin$^{-/low}$Sca-1$^+$ hematopoietic stem cells from congenic C57BL/6 mice (H2^b, CD45.1) into lethally irradiated (8.5 Gy) BALB/c mice (H2^d, CD45.2). Phenotypic T cell recovery was then followed weekly in the first 8 weeks and then once every other week until day +100 after transplantation. In this model, the origin of different T cells can be distinguished by flow cytometry (donor CD62L$^-$ T cell-derived: H2D^{b+}CD45.2$^+$, donor stem cell-derived: H2D^{b+}CD45.1$^+$, host-type: H2D^{b-} CD45.2$^+$). Stem cell-derived T cells could not be detected in peripheral blood in any recipient during the first month following transplantation. By day +35, stem cell-derived CD4$^+$ and CD8$^+$ T cells became detectable in mice receiving only purified stem cells. However, the addition of CD62L$^-$ cells into the graft dramatically increased stem cell-derived CD4$^+$ and CD8$^+$ T cell numbers in peripheral blood (Table). This effect lasted through day +49. By day +56, stem cell-derived T cell numbers became equivalent in both groups. These data demonstrate that CD62L$^-$ T cells can accelerate new T cell development from hematopoietic stem cells presumably through thymopoiesis. Mature T cells could not mediate this effect in syngeneic hosts, suggesting that this is an allogeneic effect. CD62L$^-$ T cells may be mediating the effect through enhancing the level or speed of stem cell engraftment since CD62L$^-$ T cells also imporved total white cell recovery. In conclusion, our data suggest that CD62L$^-$ memory T cells represent a unique population of T cells which do not cause GVHD but are capable of promoting new donor T cell generation from hematopoietic stem cells after allogeneic hematopoietic cell transplantation.

Table. *Allogeneic CD62L$^-$ T Cells Promote New T-cell Regeneration from Hematopoietic Stem Cells*

Groups	CD62L$^-$ T-cell–derived	(cells/μL Blood)	Stem cell–derived	(cells/μL Blood)
	CD4$^+$	CD8$^+$	CD4$^+$	CD8$^+$
Stem cell only	3 ± 1	1 ± 1	104 ± 84	18 ± 17
stem cell + CD62L$^-$ T cell	32 ± 18*	50 ± 8*	390 ± 141*	97 ± 24*

*P <.01, compared with stem cell only. Data were from day +35.

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T CELL DEPLETED HOSTS ARE UNIQUELY SUSCEPTIBLE TO TOLERANCE INDUCED BY NON-PROFESSIONAL APCS AND MEDIATED BY IL-10 PRODUCING CD8+ T CELLS

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Background: Emerging evidence indicates that the immunobiology of T cell depleted (TCD) hosts differs from normal hosts. We observed increased susceptibility to tolerance after T cell depletion and hypothesize that mechanisms at work in this model are relevant for induction of immune responses following BMT. **Methods:** Thymectomized, TCD C57BL/6 female mice reconstituted with naive T cells were immunized with male dendritic cells (DC) or male splenocytes. T cell responses to HY were measured using skin graft rejection, tetramer and ELISPOT. Male skin graft rejection correlated with expansion of HY-reactive CD8 cells and IFNγ production in response to HY by CD4 and CD8 cells. **Results:** TCD recipients of male DC generate HY specific immunity but not recipients of male splenocytes. TCD, male splenocyte immunized animals are tolerant to HY since attempts to sensitize with male DC were unsuccessful. Splenic B cells were necessary and sufficient to induce this tolerance. B cell induced tolerance to HY in TCD hosts could not be reversed by restoring normal T cell number through placement of a thymic graft or adoptive transfer of new naive T cells indicating immune suppression. CD8 T cells, but not CD4 T cells, were sufficient to mediate suppressive tolerance to HY. T cells from IL10-/- mice could not mediate tolerance whereas TGFβ neutralization had no effect. Thus, residual CD8 cells induce suppressive tolerance to HY after encounter with male B cells. Since TCD hosts have increased IL7, we hypothesized that IL7 might play a role in T cell depletion-induced tolerance. Indeed, neutralization of IL7 and IL7Rα at the time of T-B cell encounter prevented tolerance in the majority of animals. **Conclusions:** Although IL7 induced homeostatic expansion in TCD hosts is now widely appreciated as a mechanism which enhances immune responses to low affinity and self antigens, the factors which prevent autoimmunity in this setting remain unclear. These results give rise to a model wherein increased IL7 in TCD hosts prevents autoimmunity by inducing CD8/IL10 mediated suppressive tolerance to antigen presented on non-professional APCs. Therefore TCD hosts are uniquely susceptible to tolerance induction during T cell regeneration due to stringent APC requirements. While this may prevent autoimmunity during T cell regeneration, it may also limit the success of immune based therapies directed toward infectious and neoplastic antigens in clinical situations involving T cell depletion.