cell populations without changes in cell division (as measured by Ki67 expression) and recent thymic emigrants (as measured by the presence of CD44/CD62L double-positive cells) were observed with 4-week courses of IGF-1 treatment. Significantly, these changes were not observed in similarly treated thymectomized mice, indicating that effects of IGF-1 on peripheral T cell populations resulted primarily from enhanced thymic output. IGF-1 administration also affected thymic epithelial cell (TEC) turnover as measured by BrdU incorporation, with observable shifts in TEC populations in the thymic cortex and medulla as enumerated by flow cytometry. Immunohistochemistry of extracellular matrix (ECM) in IGF-1 treated young, aged, and RAG KO mice were significant for changes in laminin distribution. Finally, mice lacking IGF-1 receptor (IGF-1R) signaling on T cells were generated through T cell specific cre-mediated deletion of the IGF-1R high-affinity binding site (LCK-cre-loxIGFIR). Compared to wild-type littermates, LCK-cre-loxIGFIR mice exhibited a decrease in the number of CD4+ CD8+ thymocytes, thymic TREC, splenic naive T cell populations, and splenic TREC. These results demonstrate: (1) IGF-1 enhances the recruitment of thymocyte precursors, expands thymocyte subpopulations, and increases thymic output; (2) IGF-1 affects TEC turnover and ECM distribution, suggesting a mechanism by which IGF-1 influences thymocyte development; and (3) IGF-1R signaling is required for the maintenance of normal thymocyte and peripheral T cell populations. Together, the results support the concept of the use of neuroendocrine growth factors such as IGF-1 in preserving and/or enhancing recovery of thymic function following HSCT.

226 ANTI-MINOR HISTOCOMPATIBILITY ANTIGEN SPECIFIC CD8 MEMORY CELLS RESPOND DIFFERENTLY TO ANTIGEN AND ANTIGEN-INDEPENDENT ACTIVATION IN THE SPLEEN AND MARROW COMPARTMENTS FOLLOWING HCT

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We are interested in studying how CD8 memory cells respond in recipients following progenitor cell transplants. Antigen-specific CD8 TM against the immunodominant H60 antigen in B6 mice contribute to resistance against MHC-matched BALB.B marrow. To examine CD8 TM responses to this antigen, B6 mice were sensitized 2× with BALB.B antigen (B6.BALB.B) and 1, 5, and 22 days later, H60-specific CD8 TM in the spleen and marrow were analyzed for degranulation as assessed by CD107a expression. One day following antigen administration by foot pad injection, degranulation by CD8 TM was equivalent (~90%) in host spleen and marrow. Five days later, degranulation of splenic H60+ CD8+ TM (60%) was 3× higher (22%) versus cells in the marrow compartment. These levels decreased to 25% and 12%, respectively, in the spleen and marrow 3 weeks post antigen boost. These results indicated that CD8 TM effector-like activity is induced asymetrically in the 2 compartments and this activity may be differentially regulated in these locations. We then compared MiHA-specific CD8 TM in the marrow and spleen after antigen-independent (lymphopenia) activation. Highly enriched (>95% pure) CFSE labeled or unlabeled donor splenic CD8 T cells from B6.BALB.B mice were adoptively co-transplanted with syngeneic marrow to ablative conditioned (850 cGy) syngeneic hosts. Three days post-transfer, donor CD8 TM in recipient marrow and spleen were analyzed for CFSE dilution and degranulation including H60+ CD8 TM. Donor H60+ CD8+ TM in the marrow underwent multiple cycles of division in contrast to the spleen, indicating that the marrow compartment provided superior support for this initial homeostatic proliferation. Interestingly, 92% of donor H60+ CD8+ TM in recipient spleens expressed CD107a compared to 42% in the marrow compartment. In total, these findings indicate that whereas the marrow supported greater homeostatic proliferation of donor CD8 TM versus the spleen, the latter compartment was the preferred site for effector-like activity induced by lymphopenic conditions. These findings underscore the importance of hematopoietic compartments in shaping the diversity and differentiation of allo-specific CD8 TM responses in the transplant setting.

227 IMMUNE MONITORING WITH iTAG™ MHC TETRAMERS FOR PREDICTION OF RECURRENT OR PERSISTENT CYTOMEGALOVIRUS (CMV) INFECTION IN ALLOGENIC STEM CELL TRANSPLANT (SCT) RECIPIENTS: A PROSPECTIVE MULTICENTER CLINICAL TRIAL


Background: CMV infection is an important cause of morbidity and mortality in SCT recipients despite the introduction of routine post-transplant virologic monitoring and the use of potent antiviral agents. This prospective multicenter study evaluated the use of tetramers in monitoring CMV-specific T cell recovery following allogeneic SCT to predict patients at risk for CMV-related complications. Methods: Patients were tested every 2 weeks and monitored for up to 1 year post-transplant. iTAg™ MHC Tetramers (Beckman Coulter, San Diego) were used to enumerate CMV-specific CD8+ T cells by flow cytometry using a single-platform absolute counting method. The following tetrabodies were included: pp50: A=0.010 VTEHDTLY; pp65: A=0.201 NLVPVMATV, B=0.070 TPPRTGGGAM, B=0.301 IPSNVHYI; IE-1: B=0.080 ELRRKMMYM. All patients underwent weekly surveillance by pp65 antigenemia or DNAemia with preemptive antiviral therapy. Results: Data were analyzed for 83 CMV-seropositive recipients with 3 or more tetramer values. Median follow-up was 9 months (range 2–12). Delayed recovery of CMV-specific CD8+ T cells (<7 cells/mL in all blood samples during the first 65 days post-transplant) predisposes patients to CMV-related complications (Table 1). These patients are 2.6 times more likely to develop recurrent or persistent CMV infection, 4.8 times more likely to develop CMV disease, 2.4 times more likely to develop fatal complications, and 2.2 times more likely to develop one of more of these outcomes than patients showing rapid recovery. Rapid recovery (>7 cells/mL in any blood sample during the first 65 days post-transplant) was associated with protection from CMV-related complications. Inter- and intraassay variability of the assay was ≤8% and ≤8%, and results were available in 3 hours. Conclusions: CMV tetramer-based immune monitoring, in conjunction with virologic monitoring, can be an important new tool that permits clinicians to assess the period of risk of CMV-related complications and to make appropriate preemptive therapeutic choices in managing allogeneic SCT patients. For high-risk patients with delayed immunologic recovery, virologic monitoring, CMV tetramer monitoring, and other preemptive strategies should be continued beyond 100 days post-transplant. For low-risk patients (>7 tetramer-positive cells/mL), studies are needed to determine how long virologic monitoring should be continued or if preemptive therapy may be modified or instituted at higher viral load levels (Table 1).

Table 1. Relative Risk

<table>
<thead>
<tr>
<th>Composite Mortality (one or More Related Outcomes)</th>
<th>Recurrent or Persistent CMV Infection</th>
<th>CMV Disease</th>
<th>Transplant-Related Mortality</th>
<th>Delayed recovery of CMV-specific CD8+ cells (&lt;7 cells/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6</td>
<td>4.8</td>
<td>2.4</td>
<td>2.2</td>
<td>p = .02</td>
</tr>
</tbody>
</table>

228 EFFECTS OF THYMOLGBULIN ON LYMPHOCYTE L-SELECTIN-MEDIATED ADHESIVE INTERACTIONS

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Antithymocyte globulins (ATG) such as rabbit ATG (Thymoglobulin) are immunosuppressive agents known to deplete T cells and there is increasing evidence that they may also affect T cell migration. In this study, we sought to determine if Thymoglobulin affects L-selectin-dependent lymphocyte binding to lymph node high endothelial venules (HEV), the first step in migration of lymphocytes to lymph nodes. Human peripheral blood lymphocytes were collected by Ficoll centrifugation of citrated blood and T cells were isolated by magnetic bead separation. T cells were exposed to Thymoglobulin at 10, 20, 30, 40, and 50 ug/ml for 30 minutes at 4°C; controls consisted of purified rabbit immunoglobulin at identical concentrations. The capacity of T cells to engage HEV was measured by Stamper-Woodruff assay. Additionally, parallel plate flow chamber studies were performed under physiologic shear stress conditions to measure T cell binding to L-selectin ligands expressed on the hematopoietic cell line, KG1a. Incubation of T cells with Thymoglobulin led to a dose-dependent decrease in binding to HEV, with complete abrogation at 50 ug/ml. Similarly, Thymoglobulin blunted L-selectin-mediated binding interactions in parallel plate flow studies. Flow cytometry, performed using a variety of anti-L-selectin mAb showed, importantly, that incubation of T cells with Thymoglobulin resulted in an increase in the percent marker+ cells and the mean channel fluorescence for L-selectin epitopes targeted by mAbs TQ-1 and LAM1-116, with no change in the levels of the DREG36 epitope. These changes were specific for L-selectin, as there were no changes in expression of VLA-4 using the same conditions. Importantly, as shown by analysis of Thymoglobulin immunoprecipitates of bionylated T cells, Thymoglobulin does not directly recognize L-selectin. These findings indicate that the effects of Thymoglobulin on L-selectin function are not related to decreased surface expression, but appear to be related to changes in L-selectin topography affecting L-selectin’s capacity to engage its ligand(s). Further studies are in progress to define the nature of these changes and how modulation of L-selectin topography is achieved by Thymoglobulin. Operationally, changes in L-selectin function could have profound implications in the ability of circulating lymphocytes to enter lymphoid tissues, inhibiting appropriate antigen-recognition and subsequent generation of immune responses.

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APOTOTIC DONOR LEUKOCYTES INHIBIT TOLERANCE AND LIMIT MIXED CHIMERISM INDUCED BY CD40-CD154 BLOCKADE IN ALLOGENEIC BONE MARROW TRANSPLANTATION
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Background: "Mini" transplants using non-myeloablative conditioning can avoid early post-transplant toxicity associated with myeloablative conditioning prior to allogeneic hematopoietic progenitor cell transplantation (HPCT), but graft-versus-host-disease (GVHD) and graft rejection remain clinical problems. We previously found that low-dose busulfan conditioning and costimulatory blockade using anti-CD154 monoclonal antibody (mAb) were sufficient to establish stable mixed chimerism without GVHD when transplanting moderate doses of T cells into marrow (TCDBM) from MHC fully mismatched donors (Adams et al, J Immunol 167:1103, 2001). Additionally, several groups have shown that apoptotic cells can contribute to immune tolerance in HPCT. Therefore, in an effort to enhance donor chimerism and graft-versus-leukemia activity, we evaluated the ability of different parameters of donor lymphocytes to effect and facilitate stable donor chimerism when administered prior to the BM graft.

Methods: In B6→B10BR and BALB/c→B6 MHC fully mismatched transplant pairs, donor lymphocytes were treated ex-vivo with different immunosuppressive regimens (fludarabine, psoralen-UVA, γ-irradiation) and used as tolerizing DLI 6 days post-BMT as described previously (TCD4-CD154). CD34 subsets were also administered with low-dose busulfan conditioning. We also compared the use of donor lymphocytes that had been enriched for, or depleted of, T cells or CD11b+/CD11b− subsets as tolerizing DLI in the same "mini"-transplant setting. Results: Long-term mixed-chimerism without GVHD was enhanced by pre-transplant administration of viable allogeneic splenocytes (Table 1). Unexpectedly, the use of apoptotic/necrotic donor splenocytes resulted in diminished donor engraftment. Purified splenic T cells more potently enhanced donor chimerism compared to unfractionated splenocytes, T cell depleted splenocytes, or the CD11b+ and CD11b− fractions of donor splenocytes. In a leukemia model, the survival of recipients with mixed-chimerism following low-dose busulfan and co-stimulatory blockade was enhanced by viable post-transplant donor T cell infusions. Conclusions: Host tolerance of donor cells following non-myeloablative conditioning and co-stimulatory blockade is an active process enhanced by viable donor immune cells. The diminished donor chimerism obtained with apoptotic/necrotic donor splenocytes suggests that dying cells interfere with the donor-specific tolerance generated by anti-CD154 mAb.