Conclusions: Progressive improvement in cutaneous manifestations of cGVHD was observed following a 24-week course of ECP in pts who previously had exhibited no clinical response or worsening of cGVHD while receiving standard immunosuppressive therapy with corticosteroids. ECP improved skin GVHD and allowed for tapering of corticosteroid doses to "low risk" levels of <10 mg prednisone-equivalents/day.

406 NON-HEMATOPOIETIC ANTIGEN BLOCKS EARLY MEMORY IMPRINTING OF GRAFT-VERSUS-HOST REACTIVE CD8 CELLS
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Donor T cell alloreactivity can be co-opted to deliver a graft-versus-tumor (GVT) response following blood or bone marrow transplantation (BMT). However, the major reason for treatment failure following BMT is tumor recurrence, suggesting a long-term failure of GVTH in this study, we have considered the role of non-hematopoietic antigen in determining the fate of donor CD8 cells in a model of delayed DLI to partially MHC-mismatched chimeras, where antigen was either expressed ubiquitously or restricted to the hematopoietic compartment. We observed that donor CD8 cytotoxic and cytokine responses were poorly sustained (at >8 weeks) in the presence of non-hematopoietic antigen and this was associated with a failure to establish a central memory (TCM) population. This effect occurred at two distinct levels. Firstly, residual donor CD8 cells demonstrated a classic PD-1highCD127low 'exhaustion' signature and consistent with this, CD8 function was partially restored by blockade of the PD-1 pathway. Secondly, during the initial phase of the primary response (at day 5-8 following transfer of CFSE-labelled splenocytes), we observed that the proportion of post-mitotic, CFSE-bright CD8 cells expressing a CD4-brightCD62L+bright phenotype was much lower in chimeras where antigen was ubiquitous. These findings suggested the possibility that early CD8 encounter with non-hematopoietic antigen might severely disable memory precursor formation during the initial phase of the response. In order to test this concept, we purified CD8 T cells using congenic markers from the two sets of chimeras 14 days after initial T cell transfer, and then pumped them in secondary, antigen free hosts. After 21 days, we tested recall responses to alloantigen. We found that priming in the presence of non-hematopoietic antigen severely impaired the establishment of recall immunity in the absence of antigen. Furthermore, while a significant proportion of CD8 T cells primed in the absence of non-hematopoietic antigen had a (TCM) phenotype in secondary hosts, this population was almost entirely absent when initial priming occurred in primary hosts where antigen was ubiquitous. These data demonstrate that alloantigen within non-hematopoietic tissues is not ignored, but rather actively inhibits both the formation and maintenance of CD8 T cell memory. While these effects may lessen the risk of GVHD, they might also impair the durability of the GVTH response.

407 TRAIL/DR5 INTERACTIONS ARE IMPORTANT FOR MEDIATING THYMIC DAMAGE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION
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Thymic GVHD (tGVHD) is associated with prolonged immunodeficiency. We previously found that thymic output after allo-BMT is directly related to thymus size, and inversely related to donor T cell dose and GVHD severity. Additionally, radiation-containing preparative regimens upregulate the death receptors Fas and DR5 on thymic stroma and decrease expression of eFLIP, thus sensitizing the thymus to GVHD. Finally, very few donor alloreactive T cells are sufficient to cause tGVHD via the Fas/Fas ligand (FasL) and TRAIL/DR5 pathways. Here, we performed allo-BMT in MHC-mismatched and MHC-matched minor antigen-disparate model systems, and demonstrated the exquisite sensitivity of the thymus to as few as 1-2.5 x 10^5 donor T cells, which mediated tGVHD without significant clinical GVHD. Additionally, tGVHD is partially reversible in our models (contingent on low T cell dose), such that mice with GVHD exhibit a partial recovery in thymic cellularity late (day 60) post-transplant.

We further studied the role of TRAIL in tGVHD, by asking whether alloreactive T cells and GVHD or conditioning-associated inflammation were strictly required for TRAIL/DR5-mediated damage. We treated recipients of T cell-depleted allo-BMT with mDR5-1 agonistic antibody either in the "early" peri-transplant period, or "late," in the second week post-transplant.

Allo-BMT recipients treated "early" with mDR5-1 had significantly decreased thymic cellularity and splenic BM-derived T cells as compared with controls. Furthermore, we observed similar BM cellularity and BM-derived lineage 'sea-1' ('eikit') (LSK), in all the absence of donor alloreactive T cells or GVHD. We observed similar results with mice treated with "late" mDR5-1, indicating the continued sensitivity of the thymus to TRAIL throughout the post-transplant period, and that GVHD and/or conditioning-associated cytokines are not required to enable TRAIL-mediated damage to the thymus. Furthermore, we observed that on day 28 after T cell-depleted allo-BMT, only 1-2% of donor thymocytes expressed DR5, suggesting that mDR5-1 (and potentially TRAIL) mediate their effects on thymic cellularity and function primarily via an indirect mechanism. We conclude that the thymus is highly sensitive to GVHD and endures severe damage at low levels of systemic GVHD. Furthermore, we provide significant additional mechanistic insight into the temporal, cellular, and inflammatory requirements for TRAIL/DR5 mediated damage to the thymus.

408 P-SELECTIN REGULATES LEUKOCYTE TRAFFICKING AND EXPERIMENTAL GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

P-selectin is found on the surfaces of mature endothelium and interacts with multiple leukocyte ligands. We found that P-selectin-/- recipients of allogeneic bone marrow transplantation (allo-BMT) had significantly less GVHD mortality and morbidity, as well as decreased GVHD of the skin, liver and small bowel. This was associated with diminished infiltration of alloactivated T cells into the Peyer’s Patches and small bowel, coupled with increased numbers of donor T cells in the spleen and secondary lymphoid organs (SLO) on day 14 and day 35 post-transplant. However, donor alloreactive T cells in WT and P-selectin-/- allo-BMT recipients had similar alloactivation and apoptosis, and donor alloactivated T cells from WT and P-selectin-/- allo-BMT recipients with GVHD showed similar proliferation in vitro in a mixed leukocyte reaction, suggesting that the inflammatory environment in WT and P-selectin-/- recipients was comparable.

P-selectin glycoprotein ligand 1 (PSGL1) is the best-described P-selectin ligand, and we then tested the role of PSGL1-/- donor alloreactive T cells in mediating GVHD. To our surprise, allo-BMT recipients of WT and PSGL1-/- donor T cells had comparable survival and clinical GVHD scores, and further analyses on day 14 post-transplant revealed similar numbers of donor alloactivated T cells in the spleen, liver, mesenteric and peripheral lymph nodes, and Peyer’s Patches. Additionally, WT and PSGL1-/- donor T cells had comparable proliferation as measured by CFSE dilution, and comparable alloactivation in vivo as determined by levels of CD25, CD44, and CD69.

We then asked whether PSGL1-/- T cells might display other P-selectin ligands. Flow cytometric analyses of T cells from non-transplanted PSGL1-/- mice, and analyses of PSGL1-/- alloactivated T