examined the role of APC (monocytes and B cells) in presentation of Asp to T cells by comparing proliferative responses of APC-depleted and non-depleted samples. Although proliferation was greater in the presence of APC, depleted samples showed significant proliferation (SI 2-30) indicating that Asp directly stimulates T cell proliferation. These results show a moderately strong CD4 and CD8 T cell response to Asp that shows some TCR Vβ restriction. The T cell proliferative response to Asp appears to be partly mediated by a non-classical APC-independent pathway. These findings should facilitate the generation of Asp-specific T cells for adoptive immunotherapy.

<table>
<thead>
<tr>
<th>% Cytokine+ Cells (median, range)</th>
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<tbody>
<tr>
<td>T Cell Subset</td>
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<tr>
<td>CD4</td>
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<td>CD8</td>
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<td>CD4</td>
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<td>CD8</td>
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**TH1 AND TH2 CYTOKINE SYNTHESIS BY T CELLS OF RECIPIENTS OF G-CSF MOBILIZED ALLOGENIC PBSC**

Lee, B.; Korkling, M.; Gao, H.; Shen, D.; Martinez, M.M.; Choumplin, R.; Renben, J.M.; Anderson Cancer Center, Houston, TX.

Transplantation of G-CSF-mobilized PBSC is associated with less acute GVHD (aGVHD) and improving engraftment despite much larger numbers of T cells in unmanipulated PBSC preparations than in bone marrow grafts. As survival of the graft is dependent on the Th1/Th2 cytokine balance, we measured the syntheses of Th1 (IL-2, TNF-alpha, and IFN-gamma) and Th2 (IL-10) cytokines by CD4+ and CD8+ T cells in vitro following activation with phorbol ester (PMA) and Staphylococcus enterotoxin B (SEB) at 2, 4, 6, and 8 weeks, post-transplantation and analyzed the cells synthesizing cytokines by 4-color flow cytometry. Typically, the number of CD8+ T cells that synthesized TNF-α, IFN-gamma, and IL-2 following activation with PMA and SEB steadily increased with engraftment. The number of CD4+ T cells that were activated with PMA also steadily increased with engraftment; however, the number of SEB-activated CD4+ T cells that synthesized IL-2 peaked at 4 weeks, post-transplantation and declined thereafter. By comparison, the number of CD4+ and CD8+ T cells that synthesized IL-10 in response to activation by PMA and SEB peaked at 4 weeks and declined precipitously by week 8, post-transplantation. Since only one patient developed GVHD that resolved during the monitoring period, the increased syntheses of Th1 cytokines by activated CD4+ and CD8α T cells after engraftment and at a time when G-CSF maintenance was no longer provided is indicative of the immune competence of the engrafted cells and their propensity for producing Th1 cytokines upon activation, an event that could potentially lead to the development of GVHD.

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**RAPID RECONSTITUTION OF SERUM ANTI-PRP ANTIBODY LEVELS IN RECIPIENTS OF ALLOGENIC MARROW TRANSPLANTATION**

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As immediate survival of the transplant recipients improves, prevention of early and late post-transplant infectious complications plays a major role in long-term outcome. Timely re-immunization for pediatric stem cell transplant (HSCT) recipients may prevent devastating complications of vaccine-preventable diseases. Immune responses to polysaccharide vaccines (H. influenzae type b, pneumococcal and meningococcal) have been suboptimal early post-transplant. Polybonylribitol phosphate (PRP) is an essential component of bacterial capsular polysaccharides, such as of Streptococcus pneumoniae or Hemophilus influenzae. Natural antibodies to the polysaccharides develop as a result of the antigenic crossreactivity with E.coli strains. To evaluate the time and pattern of anti-PRP titers in pediatric patients after allogeneic related HSCT, 7 patients were evaluated 6-12 months post-BMT. Two patients had a history of acute graft versus host disease, however all patients were off immunosuppression at the time of evaluation. Titers were measured by enzyme-linked immunosorbent assay (ELISA) at Children's Hospital Los Angeles; CA. Serum anti-PRP-Pt titer varied from 16 to 900 pg/ml, median 417 pg/ml. Anti-PRP titers were higher (200-900 pg/ml, mean 900 pg/ml) in patients with non-malignant diseases compared to patients with malignancies (16-228, mean 48 pg/ml). There were no significant correlation with the T and B cell counts and T cell function. Non-protective anti-PRP levels (less than 100 pg/ml) at 1 year after HSCT were observed in two AML patients. In one low anti-PRP titers were associated with non-detectable anti-tetanus, anti-diphtheria antibody titers and low B cell count. In this study we observe rapid reconstitution of the serum anti-PRP titers in 5 of 7 recipients of allogeneic BMT. Currently we are studying the predictability of serum anti-PRP levels with regard to functional responses to vaccination post-BMT.

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**ANTI-CD3 STIMULATED T REGULATORY CELLS DO NOT INHIBIT SYNGENIC BONE MARROW PROGENITOR CELL PROLIFERATION**

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CD4+CD25+ T (T reg) cells with regulatory function are being considered for use to control GVHD and support successful allogeneic bone marrow transplantation. T reg cells are present in the spleen and bone marrow and have been reported to produce cytokines including TGFβ. Since encounter with antigen post-BMT can activate Treg populations, the presence of such cells in recipient lymphohematopoietic compartments raises the possibility that TGFβ or other cytokines could affect progenitor cell activity. Low density (LD) B6 bone marrow cells (5x10⁶/well) were supplemented with rmIL3 to induce proliferative responses in 72 hour cultures. Highly enriched Treg populations were then produced from spleen and lymph nodes of B6 (H-2b) mice. When added to cultures containing purified syngeneic CD4+CD25+ T cells (5x10⁶/well), syngeneic accessory cells (5x10⁵/well) and anti-CD3 mAb (15%), the Treg cells (1:1) effectively inhibited (>70%) the proliferative response by the CD4+CD25+ responding cells. To examine the ability of Treg cells to regulate BMC proliferation, CD4+CD25+ T cells were cultured with LD BMC and syngeneic accessory cells in the presence of anti-CD3 mAb. At high (10:1) Treg : LD BMC numbers, the BMC proliferative responses were not inhibited. In summary, the present in vitro findings support the notion that activation of CD4+CD25+ Treg cells during BMt will not interfere with the proliferative activity of transplanted or host progenitor populations. interestingly, when both CD4+CD25+ T cells and LD BMC were cultured together with Treg cells in the presence of IL-3 and anti-CD3, strong proliferative responses were detected in these cultures. These findings raise the possibility that CD4+CD25+ regulatory cells do not inhibit proliferative responses in the presence of IL-3 and BMC. Experiments are currently examining if T reg cells fail to suppress the response of the CD4+CD25+ T cells in these cultures.

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**SEX STEROID ABLATION BY CASTRATION ENHANCES HAEMOPOETIC RECOVERY FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION**


There is a general decline in immune function with age. This process, which involves thymic atrophy, is known to be reversed
by sex steroid ablation which also increases bone marrow (BM) and peripheral B cell numbers. Immune reconstitution is severely retarded in adult recipients of an allogeneic BMT. These experiments aimed to establish whether sex steroid ablation influenced haemopoietic recovery following allogeneic bone marrow transplantation. One day prior to allogeneic BMT, mice were surgically castrated. 14 days after BMT, bone marrow (16x10^6 ± 2x10^6) and thymic (55.4x10^6 ± 1.8x10^6) cell numbers were significantly increased in the castrated mice compared to sham controls (9.5x10^6 ± 0.3x10^6 and 25x10^6 ± 2.6x10^6). These remained elevated at day 28 (BM: 21x10^6 ± 4.0x10^6 vs. 14x10^6 ± 2.2x10^6; thymus: 72x10^6 ± 5.9x10^6 vs. 45x10^6 ± 2.9x10^6) at which time spleenic cellularity was also increased in the castrates. Thymocyte subsets were increased 14 and 28 days after BMT and castration. Thymic dendritic cell numbers were also increased in castrated mice 28 days following BMT suggesting a possible role in graft acceptance. BM precursors and developing B cells were significantly increased 28 days after BMT and castration. These central increases translated to a significant increase in donor-derived peripheral T and B cells 28 days after allogeneic BMT. Every immune-enhancing strategy carries the risk of exacerbating the development of graft-versus-host disease (GVHD). Mice were castrated at the same time as GVHD induction in an allogeneic setting. There was no significant difference in GVHD incidence or severity when comparing castrated and sham-castrated mice. We have previously shown that lymphoid recovery is enhanced in allo-BMT recipients after IL-7 treatment. The combination of IL-7 treatment and castration appeared to have an additive effect in the thynms 28 days after BMT and castration. These results indicate that castration and the resulting ablation of sex steroids enhance haemopoietic recovery following allogeneic BMT without increasing GVHD.

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**DONOR LYMPHOCYTE INFUSION (DLI) FROM A HLA IDENTICAL SIBLING TO TREAT A COMPLETE DIGEORGE ANOMALY (CDGA)**


OBJECTIVE CDGA is characterized by a profound T-cell immune deficiency, thymic aplasia, hypoparathyroidism, congenital cardiopathy and facial dysmorphism. Therapeutic attempts for this condition include thymic transplant and related BMT, with variable outcomes. DLI allows selective T-cell reconstitution in these rare patients, eliminating the need for a complete BMT procedure. However, studies on donor/recipient chimerism in the various myeloid and lymphoid fractions in these patients are limited. We have followed the evolution of molecular chimerism in these cell populations, and of the T-cell repertoire diversity in CDGA patients treated by DLI (4.10^6 CD3+ cells/kg), without stem-cell mobilization, conditioning regimen, nor GVHD prophylaxis. METHOD Molecular chimerism analysis was done by PCR amplification of repetitive DNA sequences (STR) at 2 loci enabling distinction between donor and recipient. Chimerism was determined at the level of the CD3+, CD4+, CD8+, CD19+ and CD56+ populations, obtained by flow cytometric cell sorting. Peripheral blood polymorphonuclear cells were used for myeloid lineage chimerism analysis. T-cell repertoire diversity and clonality was estimated by studying T-cell receptor gamma chain rearrangements within the CD3+ cell population. RESULTS Immune reconstitution is 100% donor at the T-cell level (CD3+, CD4+ and CD8+) and practically absent in other lineages. A net increase in donor-derived chimerism was observed in granulocytes following acute hepatic and medullary (pancytopaenia) GVHD treated with corticoids and cyclosporine. T-cell repertoire clonal diversity in relation to clinical events will be presented. CONCLUSION DLI allows rapid donor derived T-cell immune reconstitution, protection against opportunistic infections despite absence of thymus, and a low level of donor granulopoesis in this patient (up to 12 months of follow-up).

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**LYMPHOCYTE RECOVERY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: COMPARISON OF BONE MARROW AND PERIPHERAL BLOOD STEM CELL TRANSPLANTATION**


Background: Rate of lymphocyte recovery after allogeneic bone marrow transplantation has been demonstrated to be strong predictor of post transplant relapse in patients with acute myelogenous leukemia and acute lymphoblastic leukemia. Peripheral blood as a source of stem cells is gradually replacing bone marrow for allogeneic transplants as it has already done for autologous stem cell transplantation. It is not clear if the kinetics of the lymphocyte recovery after PBSCT is comparable to that seen with BMT. METHODS AND Materials: We identified 32 adult patients who had undergone allogeneic PBSCT for various hematological malignancies at Mayo Clinic, Rochester between 1984 and 2001. Sixty-four patients who underwent allogeneic BMT who were matched for diagnosis, conditioning regimen, GVHD prophylaxis, HLA match and age for comparison. The absolute lymphocyte count (ALC) measured at day 21 and 30 post transplant were obtained from the medical records. Results: We did not find any difference in the rate of lymphocyte recovery following allogeneic stem cell transplant with the use of BM or PBSCT as the source of stem cells. The median ALC on day 21 for the PBSCT group was 170 (range 0-880) and for the BMT group was 175 (range 0-1090); P = 0.1. For day 30, the median ALC was 365 (range 20-930) for the PBSCT group and 410 (range 40-1760) for the BMT group respectively, P = 0.1. Discussion: Use of peripheral blood stem cells has been associated with a faster hematopoietic engraftment compared to BM as the source of stem cells. In this study we have demonstrated that the lymphocyte recovery remains unchanged irrespective of the source of the stem cells. Therefore, it is likely that the previously published results on the prognostic value of lymphocyte recovery following BMT can be extrapolated to the setting of PBSCT with similar cutoff values.

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**EBV-ASSOCIATED LYMPHOPROLIFERATIVE DISORDER DEVELOPING AFTER CONDITIONING WITH RABBIT ATG**

Peters, E.M.; Madige, A.; Alljie, E.; Dansey, R. Stem cell Transplant, Karmanos Cancer Institute, Detroit, MI.

Post bone marrow transplant Epstein-Barr virus (EBV) associated Lymphoproliferative disorder (LPD) can be fatal in the immunocompromized host. The major risk factor for the development of EBV-LPD is ex-vivo T-cell depletion, and the in-vivo T-cell depletion steps caused by ATG and monoclonal T cell antibodies. Between March 1999 and January 2001 a total of 539 patients received allogeneic and autologous bone marrow transplants at the Karmanos cancer institute. During that same time period 3 patients median age 21 (range 13-35) received rabbit ATG as part of their conditioning regimen and developed EBV-LPD. Occurring at a median of 70 days after bone marrow transplant (range 60-90) median dose of rabbit ATG was 5mg/kg/dose (range 2.5-10). Treatment given in the 3 cases consisted of tapering immunosuppression and antiviral therapy, donor lymphocyte infusion in one case and chemotherapy in one case. We observed a close association with the use of rabbit ATG and the development of EBV-LPD. We believe that close monitoring and periodic testing for EBV when using this agent should be considered when ATG is used as a part of the transplant conditioning regimen.