characterize the relationship between immunophenotypic markers of T cell maturation and functional attributes of T cells, we optimized an 8-color, 10-parameter cytokine flow cytometry (CFC) approach and examined PBMC in 3 healthy donors. PBMC were exposed to stimuli that both bypass and signal through the T cell receptor (PMA/Ionomycin&em;P:I and staph enterotoxin B&em;SEB, respectively) and stained with CD45RA and CD27 to demarcate naïve (N, CD45RA+CD27+), memory (M, CD45RA-CD27-) and late-memory (LM, CD45RA+CD27-) CD4+ and CD8+ T cells. We assessed the production of effector cytokines (IL-2, IFNγ, TNFα and MIP1β) within the three maturation subsets. Following PMA and SEB stimulation, all maturation subsets (N, M and LM) of CD4+ and CD8+ T cells produced IFNγ. TNFα production was similarly distributed after P:I stimulation. However, after SEB stimulation, LM CD4+ and CD8+ cells produced only 2.5% and 8.5% of total TNFα, respectively. Production of IL-2 and MIP1-β was much more skewed, with LM cells contributing little to total IL-2 production after stimulation with P:I (0.2% of total, CD4; 0%, CD8) or SEB (CD4, 2.1%; CD8, 2.8%). While LM cells produced little or no IL-2, they produced the bulk of MIP1-β following stimulation with P:I (96.5%, CD4; 84.8%, CD8) or SEB (97.5%, CD4; 70.7%, CD8). Co-production of IL-2 and IFNγ in T cells has recently been postulated to be important for protective immunity. Importantly, no IL-2+IFNγ+ cells were seen in the LM compartment following P:I stimulation (CD4+ Distribution: 8.9% N, 91.1% M, 0% LM; CD8: 15.7% N, 84.3% M, 0% LM). Results following SEB stimulation were similar (CD4: 3.3% N, 94.9% M, 1.8% LM; CD8: 12.2% N, 87.8% M, 0% LM). Overall, these results suggest that: 1) T cell maturation stage does correlate strongly with functional capacity; 2) Nearly all IL-2 production occurs in N and M cells; 3) LM cells produce little or no IL-2; but most of total MIP-1β; 4) IL-2/IFNγ co-production is absent in LM cells, perhaps suggesting why late-stage skewing after SCT is associated with functional T cell impairment; and 5) Maturation stage appears to be more closely associated with functional status than CD4 or CD8 lineage. These results may have important implications for clinical studies of post-SCT immune recovery and GVHD.

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CLINICAL EFFICACY OF LONG-TERM LOW DOSE ACYCLOVIR FOR VZV INFECTION PROPHYLAXIS AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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The current study evaluated the clinical efficacy of long-term low dose acyclovir (ACV) prophylaxis for varicella-zoster virus (VZV) after allogeneic peripheral blood stem cell transplantation (PBSCT). The clinical data of transplant records since 2000 has been retrospectively retrieved from 2 transplant centers. Princess Margaret Hospital (PMH) group used ACV 400mg/day po till day 28, whlie Kyungpook National University Hospital (KNUH) group adopted ACV 800-1200mg/day po till at least 6-9 months. PMH group (n=193) was prone to more lymphoproliferative disorder, and more TBI-based regimen, while KNUH group (n=79) was prone to more mismatched PBSCT, and high risk acute leukemia patients. Out of 193 pts in PMH group, 26 patients (13.5%) received long-term ACV prophylaxis, while 73 patients (92.4%) in KNUH group received. Overall survival (OS) showed a trend of favorable survival toward PMH group (p=0.227), while no difference of OS between 2 groups was noted confined to HLA-identical sibling PBSCT (p=0.793). In PMH group, 42 cases (22%) developed VZV infection presented as localized (n=36) or disseminated involvement (n=6), while 6 cases (8%) of VZV infection was noted in KNUH group presenting as localized involvement (n=6; p=0.005). With median 26.5 months of follow-up among survivors, the incidences of VZV infection at 1 and 2 years were 19.2±3.3 and 27.0±4.1% in PMH, and 3.4±2.4 and 8.6±4.3% in KNUH group, respectively (p=0.002). When confined to HLA-identical sibling transplants, the incidence of VZV infection at 2 years was 26.4±4.3% at PMH, and 8.8±4.4% at KNUH (p=0.004). Taking into account for other potential risk factors for VZV infection in multivariate analysis, the use of long-term ACV as a time-dependent covariate was identified as the only independent risk factor for VZV infection after allogeneic PBSCT (p<0.001, HR 0.130, 95% C.I. [0.040~0.425]). Long-term use of ACV seemed to be protective from VZV infection after allogeneic PBSCT. Further prospective study on the role of low dose ACV is strongly warranted in allogeneic PBSCT setting.

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EARLY RECOVERY OF HOST T CELLS PREDICTS PRIMARY GRAFT REJECTION FOLLOWING NON-MYELOABLATIVE CONDITIONING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The incidence of graft rejection is increasing in association with an increased use of non-myeloablative conditioning regimen, alternative donors, and cord blood transplantation. We investigated a role of host T cells in graft rejection following non-myeloablative conditioning bone marrow transplantation (BMT) in an MHC mismatched B6D2F1 (H-2b/d) into B6 (H-2b) mouse model of BMT, where host T cells can be activated to kill donor cells. BMT following 6 Gy, 7Gy, and 10Gy total body irradiation (TBI) resulted in a primary graft rejection, secondary graft rejection, and engraftment of donor cells, respectively. Interestingly, expansion of host T cells is observed in the bone marrow and spleen 2 weeks after BMT, followed by a brisk increase in number of host T cells in peripheral blood one week later. CD8+ T cells isolated from bone marrow produced interferon-gamma and lysed donor cells. We then examined whether donor-derived dendritic cells (DCs) play a role in this rejection, using transgenic mice carrying diphtheria toxin receptor (DTR) under the control of the CD11c promoter, which allows selective ablation of CD11c+ DCs by injection of diphtheria toxin (DTx), as donors. In the recipients conditioned with 6Gy TBI, depletion of donor-derived DCs with DTx injection reduced the host T cell-expansion and leads to transient mixed chimerism in contrast to primary graft rejection in controls. We then tested whether such an early recovery of host T cells could be predictive of the graft rejection in 7 adult recipients of non-myeloablative cord blood transplantation. Three week post-transplant, an overshoot of host T cells was observed in 2 patients who experienced rejection, but in none of 5 patients with engraftment. These results suggest that residual host T cells are associated with graft rejection and an early recovery of host T cells may be predictive of graft rejection after non-myloablative allogeneic hematopoietic stem cell transplantation.

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CLINICAL EFFICACY OF CRYOPRESERVED DONOR LYMPHOCYTES FOR INFUSION (DLI)

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DLI are frequently employed after nonmyeloablative (mini) allogeneic marrow transplant (allo-BMT) to enhance chimerism. To ensure the ready availability of cells for DLI we cryopreserved CD3+ cells from mobilized allo-BMT donors. We report here the safety and efficacy of infusing cryopreserved CD3+ T cells for DLI (c-DLI) from G-CSF mobilized allo-PBSC donors and the overall clinical utility of c-DLI. **Methods:** Graded doses of CD3+ donor T cells from allo-PBSC (2 bags of 10×10^6 & 2 of 50×10^6/kg pt wgt) were cryopreserved in 10% DMSO using a controlled rate freezer and stored in vapor-phase liquid nitrogen until use. We reviewed patients (pts) who received allo-BMT for hematologic malignancy from 1/03 to

12/05 to assess the number of c-DLI administered and the effect of c-DLI on chimerism. **Results:** Of 135 allo-BMT, 29 DLI were administered to 21 pts (median 1 DLI/pt, maximum, 3), 19 of which were c-DLI and 13 of which were for mini-allo-BMT. A median of 10.5×10^6 CD3+ T cells /kg pt weight (range 5 - 108×10^6) was administered. No unexpected infusion-related toxicities or adverse events were encountered. Eight pts were unevaluable for response, due to full donor chimerism at DLI (4) or insufficient data after DLI (4). Ten pts received 11 c-DLI after min-allo-BMT to improve chimerism (4 NHL; 3 CLL; 2 Acute Leukemia; 1 MM). There were 6 responses in T cell chimerism (> 5% increase) among 5 pts (4 NHL; 1 CLL), 2 to full donor chimerism (2 NHL). The mean increase in chimerism after all c-DLIs was 16% (0 - 50) and among responders the mean increase in T cell chimerism was 24.5% and required a mean 64 days (range 14 – 130 days). Four of 5 responders were in CR before c-DLI and remained in CR, while 4 of 5 non-responders had evidence of disease before and/or after c-DLI (NS by Chi square). Fifteen pts received 21 c-DLI due to persistent or recurrent disease (5 AML, 3 CLL, 3 lymphoma, 1 each CML, MM, MDS, Breast). Two pts were not evaluable for response due to death within 30 days. Three pts had CRs attributable, at least in part, to DLI (1 each AML, CML, MM). **Conclusions:** Prospectively cryopreserved CD3+ donor T cells from mobilized PBSC are frequently employed for DLI after mini-allo-BMT. Thawed c-DLI are readily available when needed and are not associated with unexpected infusion toxicity. C-DLI are frequently effective in improving donor chimerism post BMT, however a prospective comparison of fresh vs c-DLI will be required to assess comparative efficacy.

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THYMOGLOBULIN STIMULATES TH2-TYPE ALLORESPONSES AND PRESERVES REGULATORY T CELL (TREG) FUNCTION IN-VITRO

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Thymoglobulin (rabbit anti-thymocyte globulin) is a potent T cell-depleting agent in-vivo. Nevertheless, its specific effect on T cells remains yet to be fully understood. When tested in-vitro at doses $\geq$ 500 μg/ml thymoglobulin completely depletes T cells. However, since thymoglobulin level in the serum of treated patients is consistent with a 100 μg/ml concentration in-vitro, we tested this dose in culture with purified T cells alone, or with allogeneic antigen presenting cells (APCs). Complement and 10% human serum were present in culture media. In 12-days long kinetics experiments, stimulation with thymoglobulin on d0, or d0 and d3, or d0, d3 and d6, induced T cell proliferation, measured by 3H-thymidine uptake, with peaks on d 4-5. Thymoglobulin also increased T cell responses when added in primary allogeneic mixed leukocyte culture (MLC). T cells pretreated with thymoglobulin for 3 d prior to MLC were hyporesponsive to alloantigen in secondary MLC and did not show cytotoxic T lymphocyte (CTL) activity, measured by 51Chromium release assay, while proliferated in response to restimulation with thymoglobulin. Supernatants of MLC with responder cells pretreated with thymoglobulin for 3 d contained high levels of IL-13 and IL-5, as opposed to control experiments with responders pretreated with PHA that showed high levels of IFNγ and TNFα. Since after MLC with responders pretreated with thymoglobulin >90% of T cells were CD4+CD25+CD27+ we tested the effect of thymoglobulin on immunomagnetically isolated CD4+CD25+ (regulatory T cells, Tregs) or CD4+CD25- cells. After 5 d of culture with thymoglobulin only CD4+CD25- cells had proliferated and both cell subsets expressed CD25, CTLA4, CD62L and GITR. However, only thymoglobulin-treated CD4+CD25+ cell expressed FoxP3 and suppressed T cell alloreactivity when added to a 3rd party MLC (42±27% inhibition). In conclusion, since we demonstrate that thymoglobulin initially drives T cell alloreactivity to Th2-type responses and preserves Tregs immunosuppressive activity, these findings may prompt new studies in the prevention or treatment of acute GVHD.

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STEM CELL TRANSPLANTATION (SCT) WITH AND WITHOUT CONDITIONING FOR PRIMARY IMMUNE DEFICIENCY (PID) IN CHILDREN: A SINGLE CENTER 25 YEAR EXPERIENCE

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Advances in SCT over the past 2 decades have expanded the spectrum of PID for which this form of curative therapy has been utilized. Failed, delayed and loss of donor engraftment and immune function have emerged as significant long-term problems in children with PID. We report the outcomes of conditioned (COND) and non-conditioned (UNCOND) SCT regimens in PID including: several forms of severe combined immune deficiency (SCID), and non-SCID conditions (Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), and Omenn syndrome) over the past 25 years. The majority of transplants in UNCOND SCID and all UNCOND non-SCID subjects were performed prior to 1999, utilized T cell depleted bone marrow from related donors, and did not include GVHD prophylaxis. The majority of transplants in COND SCID and all COND non-SCID patients were performed in 1999 or later, utilized bone marrow from matched unrelated and related donors and CD34+ selection of apheresed mobilized stem cells from mismatched related donors. Prophylaxis for GVHD was utilized in all recipients of unselected marrow and in recipients of CD34 selected stem cell products with $>5 \times 10^4$ T cells/kg. A total of 55 patients received 75 transplants with overall survivals of 64% and 82% in UNCOND and COND patients respectively. Overall survival in 26 UNCOND SCID patients with related donors was 65% vs 80% in 10 COND SCID patients. Four of 7 UNCOND non-SCID patients survived vs. 7 of 8 COND non-SCID patients. Among SCID survivors, 7, 3, and 1 COND patients and 13, 3, and 1 UNCOND patients received 1, 2, and 3 or more transplants respectively. Among non-SCID survivors all 4 UNCOND and 6 of 7 COND patients received only 1 transplant. Feeding problems and failure to thrive occurred frequently in SCID patients prior to transplantation. Significant pre transplant pulmonary disease adversely affected survival in both COND and UNCOND patients. Fatal post transplant EBV driven lymphoproliferation was seen in only 1 patient, following an UNCOND, T cell depleted bone marrow transplant from a haploidentical, EBV+ sibling. In COND patients, serious post transplant infections were related to central venous catheters, and preexisting pulmonary or GI infection. Our experience suggests that conditioning for SCID and non-SCID children with severe PID results in excellent survival and may prove to offer better long term engraftment and immune recovery.

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IMMUNOPHENOTYPIC AND PROTEOMIC CHARACTERIZATION OF CORD BLOOD (CB) $CD56^{BRIGHT}$ AND $CD56^{DIM}$ NK CELLS: POTENTIAL ROLE OF CB $CD56^{DIM}$ MEDIATING GVL EFFECT

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NK cells are characterized by absent CD3 and expression of CD56 and are classified into $CD56^{dim}$ (90%) that are primarily cytotoxic, and $CD56^{bright}$ that secrete cytokines (Shankaran et al *Nature* 2001). NK subsets carry out their respective functions based on their repertoire of NK receptors (NKRs) (Moretta et al *Annu Rev Immunol* 2001). Despite an immaturity in CB T-cell immunity, there is a similar leukemic relapse rate following UCBT vs. UBMT (Cairo et al *Blood* 1997). Unlike PB NK subsets, there is little information regarding CB NK subset function and NKR expression. We compared PB vs. CB NK subset NKR expression and protein expression. PB and CB cells were positively selected for $CD56^+$ using magnetic beads (Miltenyi) and sorted into $CD3^-/CD56^{bright}$ and $CD3^-/CD56^{dim}$ and NKR expression measured