ELISPOT assays. In order to remove these specific T cells from the entire spleenocyte population we took advantage of the PE labeling on the LYL8 tetramer. Using anti-PE microbeads and AUTOMACS separation, we have successfully depleted LYL8 tetramer positive T cells from both naive and immunized B6 spleens. Finally, we have been able to demonstrate that ex vivo depletion of tetramer positive cells from naive B6 spleenocytes prior to adoptive transfer along with TCD-BM significantly delayed the onset of lethal GVHD post BMT (see table).

We have shown that specific depletion of antigen specific T cells is a feasible and successful method for the elimination of GVHD in this model.

| Table. Clonal Deletion of LYL8-specific T Cells Prior to Adoptive Transfer Significantly Delays the Onset of GVHD |
|---|---|---|---|
| B6 Bone Marrow | N | Median Survival (Days) | P |
| B6 T Cells | 9 | 14 | 0.0055 |
| LYL8-B6 T Cells | 9 | 31 | |
| HSV8-B6 T Cells (non-specific control) | 4 | 8 | 0.0003 |

*P values compared indicated group to LYL8- B6 T cells group.

39 THERAPY FOR REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) WITH BASILIXIMAB (BA MAB) - A SELECTIVE INTERLEUKIN-2 RECEPTOR (IL-2R) ANTAGONIST

Moura, V., Bonfim, C.M., Bittenour, M.A., Setubal, D.C., Lenczeto, C., Ruiz, J., Zunis-Nieto, J., de Medeiros, C.R., Paquin, R. R.MT Center-Federal University of Parana, Curitiba, PR, Brazil

BAMab is a chimeric murin-human monoclonal antibody, which binds to the alpha chain of IL-2R found predominantly on activated cytotoxic T-cells. The IL-2 activation of RAS pathway, which activates lymphocyte proliferation, differentiation and development of a-GVHD, is inhibited by this drug through a competitive antagonism. We report our experience with BAMab as therapy for refractory a-GVHD after BMT. Thirty-four patients (pts) with severe refractory a-GVHD received BAMab from December 1998 to October, 2003. The latest 21 pts were treated as part of a prospective protocol. Because of a 7 days (d) half-life, BAMab schedule was 40mg weekly for 2-3 doses (adults) and 20 weekly for 2-3 doses (children). Age: 2-38 years (y) (M: 13 y); Sex M:F: 21/13. Unrelated BMT: 25 pts; related BMT: 9 pts. Cell source: Bone marrow: 30; Cord blood: 4. Diagnosis: AML (4 pts), ALL (4 pts), MDS (4 pts), CML (9 pts), Fanconi anemia (9 pts), AAS (3 pts), other (1pt). All pts had a-GVHD grade III-IV, manifesting from day 6-248 after transplant (M: 24 d), when in use of cyclosporin, and refractory to association with steroids (2-5mg/kg/d) for 7 to 70 d (M: 17 d). Sites of GVHD involvement: skin: 32 pts; GI: 25 pts; liver: 23 pts. The first administration day of BAMab occurred between 21-256 d after BMT (M: 42 d). Median follow-up was 196 d (range: 35-1847 d). Overall response rate was 82% (CR: 11 pts, PR: 17 pts). Two pts (8%) were not evaluated due to early death. Skin GVHD CR (complete response): 27/32 pts (84.3%), PR (partial response): 4/32 (12.5 %), NR (no response): 1/32(3.2%); GI GVHD CR: 12/25 pts (48%), PR: 8/25 pts (32%), NR: 4/25(16%), NA (not available): 1/25 (%); liver GVHD CR: 6/23 pt (26%), PR: 7/23 (30.5%), NR: 8/23 pts (34%), NA: 2/23 (8.8%). Time to first signs of response was 1-30 d (M: 7 d). Duration of response varied from 5-1103 d (M: 38 d). There were no infusion-related reactions. Estimated 5 y overall survival: 32%. Eleven (11) pts are alive, 7 pts with CR, 3 PR and 1 pt SR (with isolated skin GVHD). Main causes of death included: disseminated CMV (4 pt), fungal infection (6 pts), sepsis (8 pts), haemorrhage (2pts), and relapse (2 pts with ALL). GVHD relapses were seen on 14/34 pts (41%), but 7 pts (28%) were rescued with another therapy (MMF, PUVA, steroids). BAMab administration is safe and this drug is useful to treat refractory a-GVHD. Prospective studies are necessary to evaluate its optimal treatment schedule and efficacy.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

40 RESULTS OF THE CORD BLOOD TRANSPLANTATION STUDY (COBLT) UNRELATED DONOR BANKING PROGRAM


The COBLT banking program, initiated in 1996 with support from the National Heart Lung and Blood Institute aimed to 1) develop standard operating procedures (SOPs) for donor screening, recruitment and consenting and for collection, testing, processing, cryopreservation, and thawing of unrelated donor umbilical cord blood (UCB) and 2) build an ethnically diverse unrelated cord blood bank to support a transplantation research protocol. The program included multiple collection sites, 3 banks, a steering committee and coordinating center which developed a web based data collection system. Collections were initiated 12/97 and completed 7/2001. Screening data for 34,799 potential donors were collected from 4/98 to 3/2001. Of these 10,599 (30%) were not approached due to lack of informed consent or a COBLT exclusion criterion. A total 20,710 mothers (60%) of screened were approached and consented to participate in the program and UCB was collected from 48 % of those screened and 81% of those consented. 17,207 cord blood units (CBUs) of diverse ethnicity were collected (40% Caucasian; 17% African-American; 11% Asian; 20% Hispanic; 13% Mixed/Other). Of these, 11,096 (64%) were cryopreserved and placed in quarantine. Of these, 49% passed all eligibility criteria, were HLA-typed and entered into the COBLT search registry. The reasons for exclusion after collection were insufficient volume or cell count (64%), a positive maternal infectious disease test (8%) or a risk factor in the maternal history (8%). The mean total viable nucleated, CD34+ cells was collected from 9.3x10^6. The mean total CFU-GM count was 9.3x10^6. Relativeness between ethnicity, route of delivery, birth weight, maternal gestational age, infant gender and UCB yields in banked CBUs were examined. Higher cord blood volumes and total nucleated cell count (TNC) were obtained from C-section compared to vaginal deliveries. Units from African American donors contained lower TNC counts per volume compared to other ethnicities. UCB volume, TNC and CD34+ cell content correlated with birth weight. CD34+ content decreased with increased gestational age. The COBLT Program demonstrates that SOPs for donor recruitment and consenting, collection, processing, cryopreservation, quality assurance, and data collection can be successfully developed and implemented in multiple banks coordinated by a single coordinating center.

41 EMBRYONIC STEM CELLS AS AN ALTERNATIVE DONOR MARROW SOURCE: ENGRAFTMENT WITHOUT GVHD

Burt, R.K., Oyama, Y., Verda, L. Northwestern University, Chicago, IL

Hematopoietic stem cells (HSCs) have been considered unique in their ability to reconstitute hematopoiesis following transplantation into an immune compromised host. HSCs cannot be re-energized or revived ex vivo and must be individually collected from each donor or patient resulting in inter-patient as well as intra-patient lot
variability. In contrast, a single embryonic stem cell (ESC) line can be repetitively cryopreserved, thawed, expanded, and differentiated into various cellular components serving as a renewable and well-characterized stem cell source. We, therefore, determined whether ESCs could be used to reconstitute marrow and blood in major histocompatibility (MHC) mismatched mice. To induce differentiation toward HSC in vitro, ESCs were cultured in methylcellulose with hematopoietic cytokines, stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6). After 7-10 of culture, H2b ESC-derived cytokine induced hematopoietic stem cells (c-kit+CD34+) were isolated by flow cytometry and injected either intra bone marrow (IBM) or intravenously (IV) into lethally irradiate MHC mismatched H2b recipient mice. From two to twenty weeks after injection, the peripheral blood demonstrated increasing donor derived H2b positive mononuclear cells that included donor derived T lymphocytes, B lymphocytes, monocytes and granulocytes with or without clinical or histologic evidence of graft versus host disease (GvHD). Mixed lymphocyte culture (MLC) proliferation assays demonstrated T cell tolerance to both recipient and donor but intact 3rd party proliferative responses and interferon-γ production. Embryonic stem cells may be used as a renewable alternate marrow donor source that reconstitutes hematopoiesis with intact immune responsiveness without graft versus host disease despite crossing MHC barriers.

**IMMUNE RECONSTITUTION**

**42 ANTI-3rd-PARTY VETO CTLS DEPLETED OF HOST REACTIVE CLONES RETAIN A BROAD TCR REPERTOIRE: A POTENTIAL NEW SOURCE FOR ADAPTIVE IMMUNE THERAPY IN BONE MARROW TRANSPLANTATION**


CD8+ cytotoxic T lymphocytes (CTLs) are endowed with a powerful capacity to induce specific tolerance against their histocompatibility antigens (veto). Very recently, we developed a new approach to deplete CTLs of host-reactive clones by stimulating the donor T cells against 3rd party stimulators in the absence of exogenous IL-2. It could be anticipated that this procedure, which leads to a marked frequency reduction of anti-host clones, should also be associated with substantial narrowing of the TCR repertoire, thereby limiting the use of these veto cells for tolerance induction. In the present study we used a new technique (T-landscape) which enables qualitative and quantitative analysis of Vβ chain usage at the CDR3 length distribution level to compare the repertoire of anti-3rd party CTLs to that of the original donor. Mouse and human anti-3rd party CTLs were prepared as previously described. Mouse splenocytes of Balb/c and FVB were used as effectors and stimulators, respectively. Human PBMC from normal donors were stimulated against EBV-transformed cell lines, completely mismatched for HLA class I. DNA samples obtained from mouse and human CD8+ responder T cells before (day 0) and after initiation of culture (days 22 and 33 respectively) were analyzed for Vβ repertoire. The analysis showed that in CTLs of both human and mice, a significant amplification or reduction was evident only in particular CDR3 lengths of specific Vβ families. Thus, mouse anti-3rd party CTLs exhibited marked clonal reductions in Vβ3, Vβ5, Vβ12, Vβ13 and Vβ16. Changes in the latter one were most dramatic and it is almost oligoclonal. In contrast, only minor alterations were found in all the other Vβ families indicating a preservation of broad repertoire. Analysis of CDR3 lengths of anti-3rd party human CTLs showed similar preservation of broad repertoire along with marked reduction of particular CDR3 length clones in Vβ12, Vβ15 and Vβ21. These findings are of particular relevance to BMT, as it might enable to use anti-3rd party CTLs not only for enhancement of engraftment, but also for adoptive transfer of immunity during the early post transplant period. Furthermore, these results raise new possibilities for molecular probing of the antigens which trigger alloreactivity in mouse and in man.

**43 ADOPTIVE TRANSFER OF EX VIVO COSTIMULATED AUTOLOGOUS T-CELLS AFTER AUTOTRANSPLANTATION FOR MYELOMA (MM) ACCELERATES POST-TRANSPLANT T-CELL RECOVERY**


Ex-vivo co-stimulation of autologous T-cells using anti-CD3/anti-CD28-conjugated magnetic beads may restore T-cell responsiveness toward MM cells. 40 patients (pts) received ex-vivo co-stimulated autologous T-cells after autotransplantation. The mean age was 58 (range 43-72), 72% were male, 24% had IgA paraproteins, 12% had del 13 or complex karyotypes and the median β2m level at diagnosis was 3.32 mg/L (range 1.09-73.7). Lympohocyte collections were followed by cyclophosphamide (4.5 g/m²) G-CSF for stem cell mobilization melphalan (200 mg/m² or 140 mg/m² for pts ≥70). T-cells were cultured for 12 days with anti-CD3/anti-CD28-immobilized immunomagnetic beads & IL-2 supplementation (100 units/ml). During a run-in phase, 12 pts received T-cells post-transplant (~day +12) alone; afterwards, 28 pts participated in a 2 x 2 randomization in which they received T-cells “early” (day +12) or “late” (day +22) after transplant. We received 2 immunizations with the pneumococcal conjugate vaccine (PCV, Prevnar®) days +30 +90 or 3 immunizations (prior to T-cell collection, days +30, +90) to test immune responses. 27 pts received 1 or more PCV immunizations and there were no grade 3/4 adverse events. Anti-pneumococcal antibody T-cell response assays are underway for pts who completed the study; 33 pts received a mean of 8.11 x 10⁸ costimulated T-cells (range 1.6-11) all infusions were well tolerated. There were no delayed adverse effects except for grade 1-2 facial/upper body rashes in 6 pts (median 13 days after T-cells) and 1 episode of grade 2 conjunctivitis. At T-cell harvesting, the mean % of CD3+ cells in culture was 94.2% the mean T-cell doubling level was 5.2. Among the randomized pts, at day + 42 post-transplant (~30 days after T-cell infusion for the “early” groups), the mean CD4/CD3 count was 679/µL (95% CI, 347-1012) for the “early” T-cell recipients vs 278/µL (95% CI, 59-497) for the “late” T-cell recipients (T-cells not yet infused) [P = 0.03]. The mean CD8/CD3 counts were 1826/µL (95% CI, 1275-2376) 1105/µL (95% CI, 404-1806) for the “early” and “late” T-cell recipient respectively at day + 42 [P = 0.07]. 32 pts were evaluable for clinical responses (8 are too early). There were 16 CRs, 15 VGPRs (≥90% reduction in paraprotein levels), 10 PRs (50-90% reductions), 1 pt had no response. Post-transplant infusions of ex-vivo expanded autologous T-cells are feasible and well-tolerated may be associated with accelerated T-cell recovery.

**44 ACCELERATED IMMUNE RECONSTITUTION USING LLME TREATED DONOR LYMPHOCYTE INFUSIONS**

Filicko, J.E.1, Gross, D.1, Flomenberg, P.1, Friedman, T.1, Brunner, J.1, Dessain, S.1, Drobsky, W.2, Ferber, A.1, Kakhniashvili, I.1, Keefer-Taylor, C.2, Mookerjea, B.1, Wagner, J.L.1, Williams, A.1, Korngold, R.1, Flomenberg, N.1. 1. Thomas Jefferson University, Philadelphia, PA; 2. Medical College of Wisconsin, Milwaukee, WI

Delayed immune reconstitution is a major cause of morbidity and mortality after T-cell depleted allogeneic progenitor cell transplantation (PCT). To accelerate immune reconstitution without GVHD, we have administered escalating doses of L-leucyl-L-leucine methyl ester (LLME) treated lymphocytes (DLI) to 9 patients post CD34+ cell-enriched PCT in an ongoing phase 1 trial. LLME’s cellular toxicity occurs after its polymerization by dipeptidyl peptidase, leading to selective depletion of cells with cytotoxic effector