HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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IMPACT OF HLA-A DISPARITIES IN HSC TRANSPLANT FROM UNRELATED DONORS

Baxter-Lowe, L.A.[1], Haagenson, M.D.[2], Wang, T.[2], Spellman, S.R.[3], Maiers, M.[3], Marsh, S.G.E.[4], Fernandez-Vina, M.[5], Hurley, C.K.[6]. [1] University of California San Francisco; [2] Center for International Blood and Marrow Transplant Research; [3] National Marrow Donor Program; [4] Anthony Nolan Research Institute; [5] M.D. Anderson Cancer Center; [6] Georgetown University.

Since most HSC transplants using unrelated donors have HLA mismatches between the recipient and donor, better approaches for identifying the optimal donor amongst several mismatched candidates would be a major advance. The long-term goal of this investigation is to rank HLA disparities according to their effects on transplant outcomes. In this report, HLA-A is used as a model to characterize HLA disparities and examine outcomes for the most frequent disparities. Unrelated donor-recipient pairs (n = 4221) who received myeloablative predominantly marrow (92%)transplants for hematological malignancies (AML, ALL, CML, MDS and NHL) through the NMDP between 1990 and 2002 were studied. High resolution HLA typing was performed for HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, -DPA1 and -DPB1. When HLA-A, -B, -C and -DRB1 were matched, overall survival was the same if pairs were also matched for HLA-DQ and -DP (reference), had HLA-DP mismatches (OR 1.00, CI 0.84–1.18), or had HLA-DP and/or -DQ mismatches (OR 1.01, CI 0.80–1.29). These groups were combined to create the reference group for subsequent comparisons. Transplants with a single HLA-A disparity (n = 317) were associated with increased mortality (OR 1.32, CI 1.07–1.63). The most frequent single HLA-A mismatches were HLA-A*0201-*0205 (n = 28), HLA-A *0301-*0302 (n = 15), HLA-A *0201-*0206 (n = 15), HLA-A *0201-*6801 (n = 12), HLA-A*0101-*1101 (n = 11), HLA-A*0101-*0201 (n = 9), and HLA-A*2402-*2403 (n = 9). Considering p = 0.01 as a threshold for significance with multiple comparisons, there were no statistically significant relationships between any of these disparities and transplant outcomes. Table 1 shows the observations for the most interesting mismatches for grades 3–4 acute GvHD: A*2402–2403 (p = 0.034) and A*0201–0206 (p = 0.033). There were no significant relationships detected for engraftment, chronic GvHD, relapse, transplant-related mortality, or disease-free survival. Using these data, it is estimated that more than 15,000 subjects would be required to achieve 80% power to detect an effect on survival for a particular HLA-A disparity (assuming similar HLA-A frequencies). To overcome this barrier, a novel system for ranking HLA disparities which uses the structural basis for allorecognition is proposed.

Table 1. *Frequent HLA-A Disparities and Transplant Outcomes*

HLA Mismatch	Severe Acute GvHD	Survival
A*2402-2403	OR 4.70 (CI 0.91–30.4)	OR 3.02 (CI 0.56–16.3)
A*0201-0206	OR 3.22 (CI 1.02–10.50)	OR 2.64 (CI 0.81–8.38)

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CYTOTOXIC T LYMPHOCYTES (CTL) SPECIFIC FOR MULTIPLE VIRUSES CAN BE GENERATED FROM UMBILICAL CORD BLOOD FOR ADOPTIVE IMMUNOTHERAPY

Bollard, C.M.[1], Khalil, M.[1], Leen, A.M.[1], Cruz, C.R.[1], Decker, W.[2], Dotti, G.[1], Molldrem, J.J.[2], Rooney, C.M.[1], Heslop, H.E.[1], Shpall, E.J.[2]. [1] Baylor College of Medicine, Houston, TX; [2] University of Texas MD Anderson Cancer Center, Houston, TX.

Umbilical cord blood (UCB) transplantation is a promising alternative source of hematopoietic stem cells for patients lacking HLA-matched donors. Nearly 60% of UCB transplants to date have been performed on minority individuals for whom an unrelated donor was not available; moreover, the naïve phenotype of UCB cells is responsible for the lower incidence and reduced severity of GvHD in these patients. Still, relatively low cell numbers in UCB grafts have lead to delayed immune reconstitution and higher mortality due to infection. Reactivation of latent viruses such as CMV and EBV are particularly problematic, as is overt infection from adenovirus (Adv). Previous studies have shown that prophylactic adoptive immunotherapy with peripheral blood-derived CTL directed against EBV, CMV and Adv can effectively prevent the clinical manifestations of these viruses after hematopoietic stem cell transplant. We now hypothesize that generating virus-specific CTL from UCB for adoptive immunotherapy will restore anti-viral immunity and reduce viral infection post UCB transplant. Our aim was to generate multi-virus specific CTL from UCB mononuclear cells using a clinical-grade recombinant adenovirus type 5 vector pseudotyped with a type 35 fiber carrying a transgene for CMVpp65. With this Ad5f35pp65 vector we transduced UCB-derived dendritic cells to use as antigen presenting cells to stimulate virus-specific CTL followed by 2 rounds of weekly stimulation with autologous UCB-derived EBV-lymphoblastoid cell lines (LCL) transduced with the same vector. After 3 rounds of stimulation, 5 CTL cultures contained a mean of 87% (range 81–94%) CD8+ve and a mean of 26% (range 12–40%) CD4+ve cells. Evaluable CTL lines showed significant cytotoxicity in chromium release assays against non-transduced autologous LCL and LCL infected with the Ad5f35pp65 and Ad5f35 vectors. The observed cytotoxicity was specific because transduced and non-transduced MHC-mismatched LCL were not killed. ELISPOT assays on CTL demonstrated a mean of 260 (range 45–694) and 47 (range 0–128) numbers of cells secreting IFNγ following incubation with CMVpp65 and Adv hexon peptides respectively. No significant response to CMVIE1 peptides was demonstrated. These results show that, despite the generally naïve nature of UCB lymphocytes, multi-virus-specific responses can be expanded *in vitro*, and could potentially be used clinically in UCBT patients who develop infectious complications prior to immune reconstitution.

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ALLOANERGIZED HLA-MISMATCHED BONE MARROW TRANSPLANTATION – LOW INCIDENCE OF CLINICALLY SIGNIFICANT GVHD AND VIRAL INFECTION RESULTING IN LONG TERM DISEASE FREE SURVIVAL

Davies, J.[1], Gribben, J.[1], Brennan, L.[2], Nadler, L.[1], Guinan, E.[2]. [1] Dana Farber Cancer Institute, Boston, MA; [2] Dana Farber Cancer Institute, Boston, MA.

We have previously reported early results of a clinical study of HLA-mismatched related donor bone marrow transplantation (BMT) after ex vivo stimulation with recipient alloantigens and co-stimulatory blockade (CSB) to induce allospecific anergy (hyporesponsiveness) in donor T cells. We now report the long-term follow-up of a larger cohort of 24 pediatric and adult patients (median age 11 years (range 0.5–50) with high-risk hematological malignancies or bone marrow failure syndromes who received HLA-mismatched alloanergized BMT from related donors. After myeloablative conditioning with cyclophosphamide and total body irradiation, patients received donor bone marrow that had been incubated with irradiated recipient peripheral blood mononuclear cells and monoclonal antibodies blocking CD28-mediated costimulation. 21 of 22 evaluable patients engrafted (95%) and all achieved full donor chimerism by D + 21. No secondary graft failure occurred. Despite receiving a median of 2.8×10^7/kg CD3+ donor T cells (range 0.7–6.8), only 2/21 evaluable patients (10%) developed steroid-refractory acute graft versus-host-disease (GvHD), with only one death attributable to acute GvHD. Five of 11 seropositive patients reactivated CMV, but only one required extended antiviral treatment. No deaths were attributable to CMV or other viral infections. Only 1/12 evaluable patients (8%) developed chronic GvHD, which resolved with conventional immunosuppressive therapy. Eight recipients (33%) of full haplotype-mismatched BMT survive disease-free with no chronic GvHD and with normal performance scores with a median follow-up of 8 years. HLA-mismatched alloanergized BMT is thus associated with a low incidence of clinically significant viral infections and steroid-refractory acute GvHD. Furthermore, this strategy is associated with a very low incidence of chronic GvHD, and results in long-term failure-free survival with good quality of life in

a significant proportion of patients with high-risk hematological disease. This is the first report containing data on long-term toxicity and disease control after any strategy of donor graft manipulation to selectively reduce HLA-mismatched alloreactivity.

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FEASIBILITY OF UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION IN CONGENITAL CHILDHOOD DISEASES

Martinez, C.[1], Tolar, J.[1], Wagner, J.E.[1], Tan, P.-L.[1], DeFor, T.[1,2], Baker, K.S.[1], Orchard, P.J.[1]. [1] University of Minnesota, Minneapolis, MN; [2] University of Minnesota, Minneapolis, MN.

An HLA-matched sibling donor has been the initial choice for children requiring allogeneic hematopoietic cell transplant (HCT). However, less than 30% HCT patients have a matched related donor (MRD). In the past decade, umbilical cord blood (UCB) transplantation has emerged as an attractive alternative for patients without a MRD. Recent studies have shown the advantages of using UCB over bone marrow as an alternative graft source for children with acute leukemias. However, there is less information available regarding the utilization of unrelated UCB transplantation for children with non-malignant diseases. We report the use of an unrelated UCB myeloablative transplantation in fifty-five consecutive children with a median age of 2.6 years (range, 0.2–40.6 years) with Wiskott-Aldrich syndrome, Chediak-Higashi syndrome, hemophagocytic lymphohistiocytosis, langerhans cell histiocytosis, osteopetrosis, Diamond-Blackfan anemia, Hurler syndrome, Maroteaux-Lamy, α-mannosidosis, cerebral X-linked adrenoleukodystrophy, metachromatic leukodystrophy and globoid-cell leukodystrophy transplanted over 11.5 year period (1994–2006). Patients received grafts matched at 6 (14%), at 5 (56%), or at 4 HLA alleles (30%). The median total nucleated cell dose and the median $CD34^+$ cell dose were 5.4×10^7/kg and 4.2×10^5/kg, respectively. The median time to neutrophil recovery was 20 days (range, 10–45 days) and the incidence of neutrophil recovery by day 42 was 86%. The incidence of platelet recovery by 6 months was 73%. In the group of immune or hematological disorders, 6 of 10 patients achieved complete donor chimerism by day 21. In the metabolic disorders group, 8 of 13 patients achieved a complete donor chimerism at a median of 21 days and 3 additional patients at a median day of 95 days. In the leukodystrophy group, 6 of 12 patients achieved completed donor chimerism by day 21 and 4 patients achieved complete donor chimerism at a median of 120.5 days. The incidences of grade II-IV and grade III-IV acute GvHD were 34% and 12%, respectively. Chronic GvHD was observed in only 5% of cases. The overall survival was 62% at 2-years. These results demonstrate the usefulness of unrelated UCB as an alternate stem cell source for patients lacking an HLA matched related or unrelated donor. The use of unrelated UCB transplantation creates new opportunities in the treatment of non-malignant diseases requiring expedient HCT in order to prevent irreversible disease progression.

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OUTCOMES OF A PROSPECTIVE TRIAL OF NMDP-FACILITATED UNRELATED DONOR (UD) PBSC HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR LEUKEMIA AND MYELODYSPLASIA: COMPARABLE SURVIVAL REGARDLESS OF REGIMEN INTENSITY AND IMPROVED SURVIVAL WITH HIGHER CELL DOSES

Pulsipher, M.A.[1], Chitphakdithai, P.[2], Logan, B.[3], Leitman, S.[4], Anderlini, P.[5], Klein, J.[3], Horowitz, M.[3], Miller, J.[2], King, R.[2], Confer, D.[2]. [1] University of Utah, Salt Lake City, UT; [2] Nat Marrow Donor Prog, Minneapolis, MN; [3] Center for International Blood and Marrow Transplant Research, Milwaukee, WI; [4] NIH, Bethesda, MD; [5] MD Anderson Cancer Center, Houston, TX.

We report outcomes of 932 recipients (rcpts) of UD PBSC HCT facilitated by NMDP from 1999 through 2003 (median f/u 3.3 yrs). Indications were AML (419 rcpts), ALL (185 rcpts), CML (134 rcpts), and MDS (194 rcpts). Preparative regimens included myeloablative (MA, N = 611), reduced intensity (RI, N = 160), and non-myeloablative (NMA, N = 161). Distributions of HLA-match grade, CMV status, Karnofsky scores (KS), and donor characteristics were similar between the preparative regimens, however, fewer rcpts with advanced disease received NMA ($p = 0.035$), while more rcpts with coexisting diseases received RI and NMA regimens ($p < 0.001$). The age of rcpts receiving RI and NMA regimens was substantially higher than rcpts receiving MA regimens (median RI 56 yo, NMA 57 yo, MA 38 yo, $p < 0.001$). Optimal cell dose cutpoints for TNC, MNC and CD34+ were determined based on Martingale residuals from Cox regression analyses. For MA rcpts, CD34+ counts $>3.8 \times 10^6$/kg improved day +25 neutrophil and day +60 platelet engraftment; higher infused TNC doses ($>6.9 \times 10^8$/kg) predicted decreased grade III-IV aGVHD, while improved overall survival (OS) and reduced TRM (RR 0.55) were seen with MNC doses $>4.4 \times 10^8$/kg. For RI and NMA rcpts, OS was higher and TRM was decreased in those receiving $>3.8 \times 10^6$ CD34+cells/kg. Of note, cGVHD was not increased with higher cell doses in rcpts of any type of preparative regimen. Additional predictors of improved OS included early disease, and for MA rcpts only, HLA-matched donors, KS ≥ 90, and CsA-based GVHD prophylaxis. Three year OS and DFS of rcpts receiving MA, RI, and NMA approaches were similar (33, 35, and 32% OS; 33, 30, and 29% DFS: MA, RI, and NMA, respectively). Higher risk of relapse at 3 yrs in RI and NMA approaches (35, 37 vs. 24% RI, NMA, MA, respectively, $p < 0.001$) was offset by higher 3 yr TRM using MA regimens (43 vs. 34, 34% MA, RI, NMA, respectively, $p = 0.008$). Sub-analyses of 1) rcpts with AML-CR1, 2) rcpts with AML/MDS/CML (excluding ALL), or 3) rcpts between the ages of 40–60 with AML/MDS also showed similar survival with MA vs. RI vs. NMA approaches. In summary, rcpts of UD PBSC HCT receiving preparative regimens differing in intensity experienced similar survival. Higher cell doses resulted in more rapid engraftment, less severe aGVHD (MA rcpts), and better 3 year OS (37 vs. 18%, MA; 36 vs. 21% RI/NMA, $p < 0.001$), but did not increase the risk of cGVHD.

IMMUNE RECONSTITUTION

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PRE-TRANSPLANT ADMINISTRATION OF KERATINOCYTE GROWTH FACTOR AFFECTS PERIPHERAL T-CELL HOMEOSTASIS THROUGH INCREASED RECENT THYMIC EMIGRANT EXPORT AND AFFECTS THE COURSE OF MURINE CHRONIC GRAFT-VS.-HOST DISEASE

Chu, Y.-W., Bodnar, D., Singh, N., Gress, R. National Cancer Institute, Bethesda, MD.

Accelerated recovery of thymic function following allogeneic hematopoietic stem cell transplantation (allo-HSCT) not only provides a sufficiently broad repertoire of T-cell responses to pathogens, but also is thought to play a role in affecting the outcome of graft-vs.-host disease (GVHD) through the restoration of central tolerance and/or the production of regulatory cell populations that may blunt the effect of donor-derived alloreactive T-cell populations. Keratinocyte growth factor (KGF) has been shown in murine models to accelerate thymic function and ameliorate acute GVHD, but it is unclear whether the latter involves a thymic-dependent mechanism of increased T-cell production and/or cytoprotection of epithelial cells in target organs of GVHD. We examined the effect of pre-transplant administration of KGF in the B10.D2 into BALB/c murine model of chronic GVHD (cGVHD). KGF treated mice had significantly increased thymic function as assessed by enumeration of thymocyte populations, analysis of thymic cytoarchitecture, and enumeration of peripheral T-cell subsets and recent thymic emigrants (RTE). Significantly, enhanced export of RTE by KGF decreased peripheral T-cell homeostatic expansion and downregulated expression of activation markers, suggesting that RTE effectively compete with post-thymic T-cells for limited cytokines and contact-dependent niches post-allo-HSCT. Parallel experiments in thymectomized recipients receiving KGF exhibited no changes in cell cycle profiles or activation profiles of peripheral T-cells. Pre-transplant KGF administration improved clinical cGVHD outcomes in both thymus-intact and thymectomized recipients. However, there were no observable differences in the course of cGVHD between KGF treated thymus intact and thymectomized mice, suggesting that enhanced thymic function by KGF did not provide for any additional benefit to the cytoprotective effects of KGF. One contributing factor for this observation was