donor PBMC with PPC-pulsed DC. By week 3–4, both lines were strongly cytotoxic and produced IFN-γ (but not IL-4) in response to PPC-pulsed targets. CTL activity and IFN-γ production by donor 1 was mediated by HLA-DRB1*0301-restricted CD4+ T cells. Screening with 21 smaller peptide pools, then with single peptides from the positive pools identified the recognized peptide as TWSIDGAVVRT. A database search of peptides likely to be presented by DRB1*0301 narrowed the sequence to WSIDGAVVR (aa 174–182) (WSI). Screening of PBMC from DRB1*0301+ donors by ELISPOT revealed IFN-γ response to WSI in 5/6 donors. All donors responded to PPC. CTL activity and IFN-γ production was mediated by HLA-B*3501-restricted CD8+ T cells for donor 2 and via small pool followed by single peptide screening and a database search, 3 peptides were identified, YFKYTAAAL (aa 2–10) (YFK), LPLCSAQTW (aa 14–22) (LPL), and GTRFPQTPM (aa 192–200) (GTR). Two of the 3 peptides were also presented by B*3503, YFK, and GTR, but 0/3 were presented by B*3502 or B*3508. Supernatant from both CTL lines was toxic to *A. fumigatus* conidia in a FUN-1 assay and CTL were directly cytotoxic to *A. fumigatus* hyphae in an XTT assay. These data demonstrate the ability of Asp f16 to induce Th1-type T cell responses. Use of a pool of overlapping pentadecapeptides can prime both CD4+ and CD8+ T cells and is not limited to individuals of a given HLA type. This represents a unique approach to the prevention or treatment of IA in immunocompromised patients.

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EX VIVO EXPANDED MYELOID PROGENITOR CELLS PROTECT NEUTROPENIC MICE FROM FUNGUS INFECTION

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Our long-term goal is to develop a cell-based short-term bridging therapy to enhance resistance to pathogens and recovery from infections after hematopoietic cell transplantation or chemotherapy. The use of mature myeloid cells is limited by the large numbers of cells needed, their very short half-life, and the inability to cryostore these cells. Myeloid progenitor cells, on the other hand, can be cryostored and can result in significant protection with fewer cells. Several populations, defined by surface markers in both humans and mouse, are part of the MP pool. These include common myeloid progenitors (CMPs), granulocyte macrophage progenitors (GMPs), and megakaryocyte erythroid progenitors (MEPs). Preclinical models with murine MP, focusing on CMP and GMP, have demonstrated that a single infusion, even with fully allogeneic cells, can prevent fungus-induced mortality. To obtain sufficient cells for clinical use, we aim to derive MP from highly purified HSC ex vivo, using the inevitable myeloid differentiation that is observed during attempatients to expand HSC ex vivo to our advantage. Highly purified mouse KTLS HSC, defined as CD117+, CD90.1^{low}, Lin$^{neg/low}$, and Sca-1+, were sorted and cultured under serum-free conditions to derive MP. Using a 4-growth factor cocktail (KitL, Flt3L, Tpo, and IL-6), we observed an approximately 100-fold increase in MP relative to the number of KTLS HSC plated over a 1-week culture period. A mouse model was used to test the ability of these cells to protect mice against fungus infection. Infection was induced by intravenous injection of conidia derived from a clinical isolate of *Aspergillus fumigatus* 8 days after fully myeloablative preconditioning and HSC transplantation. These studies confirm that MP derived from HSC ex vivo retain the potential to protect severely neutropenic mice from a normally lethal challenge with *A. fumigatus*. Moreover, both syngeneic and fully allogeneic (AKR to C57BL/Ka, C57BL/Ka to BALB/c) culture-derived MPs provide effective protection. Reconstitution analysis confirms that protection does not require long-term engraftment by the MP-derived cells in vivo. These preclinical experiments indicate that large numbers of functional MPs can be obtained from purified HSC by ex vivo expansion.

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NK CELL KILLER IMMUNOGLOBULIN RECEPTOR (KIR) RECONSTITUTION AND INTERFERON PRODUCTION AFTER UNRELATED DONOR (URD) TRANSPLANTATION IS ALTERED BY THE T-CELL CONTENT OF THE GRAFT AND CORRELATES WITH ACUTE GVHD

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KIR ligand mismatched haploidentical T-cell depleted (TCD) transplants prevent AML relapse, GVHD, and graft failure, but similar outcomes are not achieved with the same strategy when T-cell- replete (non-TCD) grafts are used. We hypothesized that the variable clinical benefit is due to differences in KIR reconstitution and NK cell function affected by competing donor T cells, which may dilute the benefit of NK-cell alloreactivity. We studied cryopreserved PBLs collected by the National Marrow Donor Program Research Sample Repository from 93 URD transplant recipients (48 TCD, 45 non-TCD) and their donors, who provide perfect controls for the genetic determinants of the KIR repertoire. At day 100, all recipients had an average 4-fold increased percentage in NK cells compared with their donors. Importantly, the quality of these NK cells differed significantly based on the T-cell content of the graft. Recovered KIR expression (assessed by flow cytometry using a cocktail of 4 KIR antibodies, DX9, EB6, GL183, and FES173) more closely resembled the donor NK cells in TCD transplants (recipient:donor ratio of KIR+ NK cells 0.91 ± 0.08, n = 37). In contrast, recipients of non-TCD grafts had a significantly diminished KIR ratio of 0.63 ± 0.07 (n = 35; $P = .017$). CD94 and NKG2A were increased in recipients in both groups, and were reciprocally highest when KIR was lowest. We also tested the function of the reconstituted NK cells by measuring their IFN-γ production by intracellular cytokine staining after incubation with IL-12 and IL-18. Although donor IFN-γ was similar in both groups, recipients of non-TCD grafts had increased IFN-γ-producing NK cells compared to TCD transplants ($53.96 \pm 4.47\%$ vs $34.86 \pm 5.7\%$, n = 49; $P = .006$). Patients who developed acute GVHD had a significantly increased percentage of recipient NK cells producing IFN-γ ($51.81 \pm 3.76\%$ vs $20.79 \pm 4.76\%$; $P < .0001$) and a decreased KIR expression ratio (0.64 ± 0.06 vs 0.97 ± 0.13, n = 49; $P = .03$), suggesting a direct relationship between lower KIR expression and increased NK cell IFN-γ production. These data show that T cells in the graft alter the KIR repertoire and function of reconstituted, donor-derived NK cells after unrelated donor transplantation, which in turn affect clinical outcomes. This supports the premise that exploiting the benefit of NK-cell alloreactivity may be best realized without T cells competing for the same factors that control homeostatic expansion of NK cells.

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ENGINEERING DONOR MESENCHYMAL CELLS WITH IL-7 HASTENS NAIVE T CELL RECRUITMENT IN VITRO AND SUPPORTS IMMUNOLOGIC RECONSTITUTION AFTER HSCT IN NOD/SCID MICE

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Key players in human T-cell development after T- cell-depleted allogeneic stem cell transplants are bone marrow stroma and interleukin-7 (IL-7). We engineered human stromal cells with the IL-7 gene and studied the effects on T cells in vitro and on in vivo immunological reconstitution in NOD/SCID mice. Transduced mesenchymal cells were negative for CD45 and CD14, positive for CD90 (98.15%), CD105 (87.6%), and STRO-1 (86.7%) and stably produced IL-7 ($16.37 \pm 2SD$ pg/ml). In cocultures with T cells, IL-7 engineered stromal cells inhibited PHA-induced T cell proliferation (proliferation index, 3.6 vs 8.0 in untransduced cells and 65.8 in PHA alone), and in cocultures with immunoselected naive T cells, they maintained the CD45RA+CD45RO- naive phenotype (resting naive cell count, 4.2 times more than controls). In NOD-SCID mice, they homed to all organs (highest percentages in liver and lung; overlapping signals in spleen, thymus, bone marrow, heart, kidney, skin and gut; traces in brain). In a NOD/

SCID model, comparing transplantation of 1×10^6 CD34+ cells, cotransplantation of 5×10^5 untransduced mesenchymal cells and 1×10^6 CD34+ cells and cotransplantation of 5×10^5 IL-7 engineered stromal cells with 1×10^6 CD34+ cells, the last improved engraftment in terms of CD45+ cells and significantly increased the CD3+ cell count in peripheral blood (1.4 ± 1.6 vs 7.4 ± 3; $P < .05$), bone marrow (0.8 ± 1 vs 5.5 ± 2; $P < .05$) and spleen (0.08 ± 0.1 vs 6.2 ± 2; $P < .05$). No significant differences emerged in CD19+ cell recovery. IL-7 serum concentrations did not vary at any time point posttransplantation. These data demonstrate that IL-7-transduced stromal cells maintain the naive T-cell phenotype (CD45RA+/CD45RO−) in vitro and serve to improve immunologic reconstitution after HSCT in NOD/SCID mice, thus emerging as an ideal scaffold for hastening immunological recovery in T-cell–deficient hosts.

LATE EFFECTS/QUALITY OF LIFE

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LIMITING ALLOGENEIC BONE MARROW TRANSPLANTATION BASED ON PSYCHOSOCIAL CRITERIA: A COMPARISON OF PROFESSIONAL ATTITUDES

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Oncologists specializing in bone marrow transplantation (BMT) make decisions about who is an appropriate medical candidate for an allogeneic BMT based on clinical criteria. It is not clear how and if decisions are made as to whether a patient is an appropriate candidate based on psychosocial criteria. Although setting limits based on such criteria has been researched in solid organ transplantation, this is not the case in BMT. Presented will be results from survey research comparing responses of BMT physicians, social workers, and nurses regarding acceptability of select psychosocial screening criteria in allogeneic BMT. An IRB-approved survey was mailed to members of ASBMT (North America), AOSW, BMT Special Interest Group, and ONS BMT Special Interest Group. Respondents were asked whether they would recommend proceeding or not proceeding with allogeneic BMT in 17 case vignettes, all of in which the patient has leukemia and had a matched donor. Based on 262 physician surveys returned (40% response rate), the following are 7 case vignettes in which the majority stated that they would recommend not proceeding: active suicidal ideation (84%), current use of addictive illicit drugs (76%), noncompliance (76%), no caregiver available posttransplantation (69%), active alcoholism (61%), early onset of Alzheimer's disease (55%), and no means to pay for BMT (51%). In 10 of 17 case vignettes, most physician respondents would recommend proceeding: history of prior suicide attempts but not currently suicidal (86%), mild mental retardation (84%), major depression (84%), controlled schizophrenia (82%), daily marijuana use (82%), history of a violent felony crime (81%), borderline personality disorder (79%), tobacco smoking (79%), morbid obesity (71%), and caregiver with mental health problem (65%). These initial findings infer that whether or not physicians decide to proceed with allogeneic BMT may be contingent on the currency and acuity of psychosocial risk factors predictive of impaired patient ability for informed, competent decision-making. Responses of physicians, nurses, and social workers are compared and implications for clinical practice in BMT discussed.

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HSCT FAMILY CAREGIVER QUALITY OF LIFE INFLUENCED BY HIGH CAREGIVING STRAIN AND LOW PREDICTABILITY OF CAREGIVING

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Background/Purpose: Family caregivers (FCGs) provide much of the outpatient care for allogeneic HSCT recipients during the first year after transplantation. Despite the importance of family caregiving, there is little information about the types of care activities required during this period and the effect of caregiving role strain (CRS) on FCGs' quality of life (QOL). The purpose of this study is to describe relationships among preparedness for caregiving, predictability of caregiving, recipient function and CRS on FCG QOL. **Methods:** FCGs (N = 56) were recruited from 2 academic medical centers on the West Coast and completed a single questionnaire between 3 and 15 months (mean, 7.7 months) after family members' allogeneic HSCT. Descriptive statistics, correlation, and simultaneous regression techniques were used in this cross-sectional study. **Results:** Three types of caregiving were inductively derived from 100 activities: (1) performing usual care (eg, supervising scheduled medications, keeping house clean), (2) providing emotional support (eg, talking with HSCT recipients when sad, discussing how people respond to recipients' illness), and (3) making care-based decisions (eg, giving prn medications, noticing subtle changes). Providing emotional support was the most difficult type of caregiving activity (mean, 1.81; range, 0 = not done, 1 = easy, to 5 = very hard). FCGs had little difficulty performing usual care (mean, 0.90) and making care-based decisions (mean, 1.28). FCGs felt moderately well prepared for caregiving (mean, 2.9; range, 1–4). Together, preparedness, recipient function, predictability, and the 3 types of caregiving accounted for 37% of the variance in FCG QOL ($F6,48= 7.3$; $P < .001$). FCG QOL was uniquely predicted by predictability of caregiving ($t48 = 2.5$; $P = .02$) and strain from providing emotional support ($t48 = -1.9$; $P = .05$). **Conclusions:** During the first year after allogeneic HSCT, FCGs need ongoing assistance providing emotional support to HSCT recipients and recognizing predictability of recipients' caregiving needs.

LEUKEMIA

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EARLY ALLOGENEIC STEM CELL TRANSPLANTATION FOR YOUNG ADULTS WITH ACUTE MYELOBLASTIC LEUKEMIA IN FIRST COMPLETE REMISSION: AN INTENT-TO-TREAT ANALYSIS OF THE LONG-TERM EXPERIENCE OF THE BGMT GROUP

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We evaluated the outcome of early allo-SCT through 4 successive protocols over 17 years by an intent-to-treat analysis based on donor availability in patients younger than 45 years with CR1 AML. Of the 472 patients achieving CR, 182 had an HLA-identical sibling identified, and allo-SCT was carried out in 171 (94%) after Cy-TBI. Of the 290 patients without donor, 88% were intended (and 63% received) to receive autologous auto-SCT. With a median follow-up of 114 months, the 10-year probabilities of relapse, nonrelapse death, overall survival (OS), and leukemia-free survival (LFS) were 27% versus 55% ($P < .001$), 24% versus 6% ($P < .001$), 51% versus 43% ($P = .11$), and 48% versus 40% ($P < .03$), respectively, for alloSCT and non-alloSCT treatment. Cox analysis identified initial WBC, FAB subtypes, cytogenetic risk group, and number of induction courses to achieve CR as significant prognostic predictors. We calculated the statistical risk of each combination of these 4 factors for patients not having a suitable donor and identified 3 groups of patients with different outcome (poor, intermediate, and standard risk group). This analysis allowed us to reclassify 21% of the patients as compared with