$P = .007$). This association was observed irrespective of the type of GVHD prophylaxis given. There was no difference in chronic GVHD (18/32 [56%] vs 43/77 [56%] in the mismatched and matched cohorts, respectively; $P > .99$). Relapse rates were also similar (49% vs 53%; $P = .84$). Actuarial 1-year OS was 56% for the mismatched group and 54% for the matched cohort, with a 2-year OS of 40% and 35%, respectively ($P = .40$). Among patients mismatched at HLA-C, aGVHD incidence was 16/23 (70%), and there was a significant trend toward decreased 1 and 2-year survival (45% and 22%, vs 60% and 44% in C-matched patients; $P = .07$). There was no difference in relapse associated with HLA-C disparity. In Cox regression-adjusted for HLA-disparity, donor/recipient age, gender disparity, stem cell source (BM vs PBSC), disease status, and GVHD prophylaxis, there remained a trend toward worse survival for patients mismatched at HLA-C (HR 2.1; $P = .09$), and grade II-IV acute GVHD was the only statistically significant predictor of death (HR 4.7; $P < .001$). We conclude that single antigen/allele mismatch in HLA-A, B, and DQ is permissible for URD NST, but HLA-C disparity should be treated with caution.

41

CONTRIBUTIONS OF KIR GENOTYPE AND HLA-C-ENCODED KIR LIGANDS TO THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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A hallmark of human natural killer cells is the expression of killer-cell immunoglobulin-like receptors (KIR), which enable the detection of changes in HLA class I expression. The KIR family is highly polymorphic and characterized by interindividual differences in the number of inherited genes, which vary from 7 to sometimes 14 different genes. The aim of this study was to analyze the influence of KIR genes and HLA-encoded ligands on allogeneic SCT outcome. Statistical analyses of transplantation outcomes in unrelated HLA-matched transplantation for chronic myeloid leukemia (CML; n = 109) by univariate and multivariate analyses exhibited a significant influence of HLA-C-encoded KIR epitopes on overall survival. Whereas patients with 2 HLA-Casn80 allotypes (HLA-C group 1) had an overall survival (OS) at 6 years of 76%, patients with 1 or 2 HLA-Clys80 allotypes (HLA-C group 2) exhibited a significantly decreased OS of 54% (independent of transplant source and disease status in logistic regression analysis). Among the latter subgroup, those patients, who received a transplant from a donor with 2 HLA-Clys80 allotypes had a substantially better event-free survival (EFS = 79%) than those who received a transplant from a donor with 1 or 2 HLA-Casn80 allotypes (EFS = 40%). Next, generic typing for presence or absence of KIR genes was employed to assess the contribution of KIR genotype on SCT outcome. A subgroup of patients was defined who received grafts from donors with 1 additional stimulatory KIR gene compared to the patient's genotype (KIRS_D+1). A beneficial influence of the KIRS_D+1 constellation on OS was seen in the unrelated SCT study described earlier, as well as in a related SCT study of HLA-identical sibling donor transplantations (n = 65). Moreover, epistatic interaction was seen between KIR genes and HLA-C epitopes; among the patients with 1 or 2 HLA-Clys80 allotypes, the KIRS_D+1 group performed significantly better (OS = 83%) than the remaining patients (OS = 47%). In summary, consideration of donor/patient KIR genotypes in combination with patient-encoded KIR ligands might lead to an improvement in donor selection in SCT.

IMMUNE RECONSTITUTION

42

THE GENERATION OF DONOR-DERIVED VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES REACTIVE AGAINST CYTOMEGALOVIRUS AND ADENOVIRUS ANTIGENS FOR CLINICAL USE

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Cytomegalovirus (CMV) and adenovirus are major viral pathogens causing morbidity and mortality in immunocompromised patients undergoing allogeneic stem cell transplantation. Previous studies have shown that prophylactic adoptive immunotherapy with donor-derived cytotoxic T lymphocytes (CTLs) directed against Epstein-Barr virus (EBV) and CMV can effectively prevent the clinical manifestations of these viruses. We have extended these studies by generating CTLs from normal donor PBMCs that should restore cellular immunity to both CMV and adenovirus simultaneously. We have generated a clinical grade recombinant adenovirus type 5 vector pseudotyped with a type 35 fiber (Ad5f35pp65) carrying a transgene for the immunodominant CMV antigen, pp65. We have developed a protocol using an initial round of stimulation with autologous mononuclear cells transduced with Ad5f35pp65, followed by 2 rounds of weekly stimulation with autologous EBV-transformed lymphoblastoid cell lines (LCLs) transduced with the same vector using MOIs of 10 and 100, respectively. After 3 rounds of stimulation, 3 CTL cultures contained a mean of 86% (range, 82%-95%) CD8+ve and a mean of 9.5% (range, 5%-14%) CD4+ve cells. Evaluable CTL lines showed significant cytotoxicity in chromium release assays against autologous fibroblasts and LCL infected with a CMV vector and LCL infected with the Ad5GFP vector. The observed cytotoxicity was specific because nontransduced LCLs, transduced and nontransduced MHC-mismatched LCLs, and autologous PHA blasts were not killed. ELISPOT assays on CTL demonstrated a mean of 459 (range, 284–634) and 497.5 (range, 402–593) numbers of cells secreting interferon-gamma after stimulation with autologous PBMC stimulated with CMV and adenovirus peptides, respectively. Using MHC-peptide tetramers in a HLA-A2/B7+ve donor we have been able to demonstrate the simultaneous presence of CD8+ve cells recognizing peptide epitopes from CMV pp65 (range, 2.41%-9.08%) and adenovirus hexon (1.69%) in the CTL culture. In summary, we have developed a protocol for the efficient generation ex vivo of CTL with virus specificity for CMV and adenovirus. We are currently growing these CTL lines for clinical use in a FDA-approved protocol to restore cellular immunity to these viruses after allogeneic stem cell transplantation.

43

ASPERGILLUS FUMIGATUS-SPECIFIC CYTOTOXIC T-CELL LINES GENERATED THROUGH USE OF ASP F16 PROTEIN-SPANNING POOLS OF OVERLAPPING PENTADECAPEPTIDES

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Invasive aspergillosis (IA) fungal infection is often lethal in immunosuppressed individuals, particularly patients undergoing blood or marrow transplantation. Although first-line defense against IA is largely mediated by neutrophils and macrophages, there is evidence that Th1 type T-cell response is essential in the control of infection. We prepared 104 overlapping pentadecapeptides (11-aa overlap with previous peptide) spanning the coding region of the aspergillus allergen, Asp f16, previously shown to induce Th1-type cell responses in mice. These were used to prime T-cell responses in healthy donors. PBMC from 5/5 donors proliferated strongly to complete peptide pool (PPC)-pulsed autologous monocyte-derived dendritic cells (DC) generated by 2–3 days of culture with GM-CSF and IL-4, pulsed, then matured for 2 days with a cocktail of inflammatory cytokines. Cytotoxic T lymphocyte (CTL) lines were generated from 2 donors by weekly priming of

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.

44

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.

45

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 ± 4.47% vs 34.86 ± 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 ± 3.76% vs 20.79 ± 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.

46

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±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/