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 ± 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.

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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±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/