Average infused TNC/kg $\times 10^7$ was 2.9 (1.9–5.8) and 4.7 (0.6–9.1) for the non-cultured and cultured cells respectively, and infused CD34 cells/kg ($\times 10^5$) was 2.2 (1.1–3.4) and 55 (9.1–136) respectively. No toxicities directly attributable to the cultured product, including infusional, increased acute GVHD, or graft failure have been observed. Relatively rapid engraftment was observed in 7 of 8 patients with a median time to engraftment of 16 days (7 to 34), as compared to 25 days (16 to 48) in patients (n = 17) undergoing an identical transplant regimen here, but with 2 non-cultured CBU. Relative contribution of the expanded and non-cultured grafts over time was determined by a DNA-based assay on peripheral blood, beginning day 7 post transplant. Engrafted myeloid cells present at day 7 were derived almost entirely from the expanded unit in 7 patients. In 3 of 7, ANC > 500 was observed at days 7, 9 and 16 and was mainly derived from the expanded unit, whereas in the other 4 patients who achieved ANC > 500 at day 13, 16, 20 and 21, myeloid engraftment at day 14 was derived from the non-cultured cells. Persistent engraftment from the expanded cells has been noted in 2 patients, one through 280 days post transplant and one who is currently 125 days post transplant in whom the expanded cells continue to dominate in CD33, CD14 and CD56 sorted cell fractions. Average follow-up time is 287 days (range 56–680). One patient died on day 462 from complications of VZV myelitis; all other patients are alive and in remission. Thus, improvement in early myeloid reconstitution may result from provision of short term repopulating cells and/or of cells able to facilitate engraftment of the non-cultured unit. These studies continue with the goal of achieving consistent, rapid engraftment in recipients of hematopoietic cell transplants to decrease morbidity and mortality in the early post-transplant setting.

GVH/GVL

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TARGETED PROTECTION OF THE COLON IN THE ABSENCE OF DONOR ANTIGEN-PRESENTING CELL -DERIVED INTERLEUKIN 23 ALLOWS FOR SEPARATION OF GRAFT VERSUS HOST AND GRAFT VERSUS LEUKEMIA EFFECTS

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Separation of graft versus leukemia (GVL) and graft versus host (GVH) reactivity has been a longstanding but elusive goal in allogeneic bone marrow transplantation (BMT). Recent studies from our laboratory have shown that donor APC-derived IL-23 secretion has a critical role in mediating pathological damage in the colon during GVHD, but has no protective effect in other GVHD target organs such as the lung and liver. These results demonstrate the feasibility of regional GVHD protection and raise the question as to whether such localized protection might be a strategy to separate GVH and GVL responses. To directly address this question, we employed a novel, clinically relevant murine model of chronic myelogenous leukemia (CML). In this model, FVB animals that have the bcr/abl oncogene under the control of a tetracycline-inducible repressor are used as donor animals (i.e. CML mice). Lethally irradiated normal FVB mice were transplanted with equivalent numbers (10^7) of T cell depleted BM from B6 and FVB CML animals. Withdrawal of tetracycline from the drinking water induces expression of the bcr/abl oncogene and the development of granulocytic hyperplasia and splenomegaly in transplanted mice. Additional cohorts of animals were also transplanted with the same BM inoculum plus 3.5×10^6 T cells from either wild type or IL-$23^{-/-}$ donors to determine if GVL and GVH effects could be dissociated. Whereas mice that received adjunctive B6 T cells succumbed from fatal GVHD, animals transplanted with T cells from IL-$23^{-/-}$ mice had significantly prolonged survival and no evidence of leukemia by blood counts or pathological examination. In order to address the role of IL-23 in the GVL response directed against leukemia with more aggressive kinetics, similar studies were performed using a Balb-derived A20 leukemia cell line. Transplantation with BM and spleen cells from IL-$23^{-/-}$ donor mice resulted in significantly prolonged survival when compared to mice reconstituted with similarly composed marrow grafts from wild type B6 animals. A five–fold escalation of the A20 cell dose produced similar protective results in recipients of IL-$23^{-/-}$ marrow grafts. In conclusion, these studies show that in the absence of donor APC-derived IL-23, GVHD can be significantly reduced without loss of the GVL effect. Moreover, our results suggest that targeting of IL-23 may be a viable clinical strategy to ameliorate that severity of GVHD without abrogating a GVL response.

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FLAGELLIN, A TLR5 AGONIST, FACILITATES PRODUCTION OF FOXP3+CD4+CD25+ REGULATORY T CELLS TO MAINTAIN BALANCED IMMUNE RECONSTITUTION IN ALLOGENEIC BMT WITHOUT GVHD

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Background: Graft-vs-host disease (GvHD) and lyphopenia are the major clinical problems in allogeneic bone marrow transplantation (BMT). Immunosuppressive drugs are used to control GvHD, but immunosuppression is often incomplete and patients experience drug-related toxicities. Donor foxp3+CD25+CD4+ regulatory T cells are also effective at controlling GvHD, but are expensive and time consuming to produce. Flagellin, a bacterial protein and a TLR5 agonist, can culminate production of proinflammatory cytokines and chemokines. In this study we investigated whether flagellin could facilitate thymic production of donor BM-derived foxp3+CD25+CD4+ regulatory T cells to control GvHD and regulate post BMT immune reconstitution.

Methods: Irradiated (11Gy) CB6F1 recipient received 5×10^6 T cell depleted BM and 5×10^6 splenocytes from naive C57BL/6 congenic donors. 50 micro gm flagellin/mouse was administered i.p. 3 hours before irradiation and 24 hours after BMT. Recipients that received no flagellin were used as control. After 70+ days post transplant recipients were infected with 5×10^3 pfu MCMV i.p., sacrificed at different time points and lymphocytes were harvested from spleen and thymus for analysis. Flow cytometry was used to determine immune reconstitution, normal and regulatory T cells.

Results: All flagellin-treated recipients survived without GvHD for 66 days post transplant, while only 65% of the control mice survived and had chronic GvHD. The number of splenocytes was significantly increased in flagellin-treated recipients compared to control recipients (p = 0.0006) on day 66-post transplant. Donor spleen- and BM-derived CD4+ and CD8+ T cells were significantly higher in the spleen of flagellin-treated recipients compared to control mice. Flagellin-treated recipients had higher levels of both donor spleen- and BM-derived anti-viral CD8+ T cells in the spleen compared to control recipients. The thymus of flagellin-treated recipients produced donor spleen- and BM-derived T cells and foxp3+CD25+CD4+ regulatory T cells, while thymic functions were severely reduced in control recipients.

Conclusion: Flagellin treatment successfully reduced GvHD, improved survival, enhanced donor T-cell engraftment and produced regulatory T cells in allo-BMT. Treated recipients had brisk and persistent cellular immune responses against MCMV infection. Hence, prophylactic use of flagellin is a novel therapeutic approach to treat blood cancer patients with allogeneic BMT.

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LYMPHOCYTE RECOVERY IS A MAJOR DETERMINANT OF TRANSPLANT OUTCOME AFTER MYELOABLATIVE TRANSPLANTATION IN PATIENTS WITH MYELOID MALIGNANCIES RECEIVING MATCHED UNRELATED STEM CELL ALLOGRAFTS

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A higher lymphocyte count one month after allogeneic stem cell transplantation (SCT) is associated with better outcome in patients transplanted from an HLA-identical sibling. However, a predictive role of the day 30 post-transplant absolute lymphocyte count (LC30) in unrelated transplants is not defined. We studied the relationship between LC30 and outcome in 102 patients with myeloid leukemia receiving myeloablative SCT from matched unrelated donors. Conditioning consisted of cyclophosphamide with Busulphan (n = 61) or total body irradiation (n = 41). Immunosuppression