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 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, in a number of cases, early neutralized engraftment 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 leukaemia (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 \(\times 10^7\) 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 leukaemia by blood counts or pathological examination. In order to address the role of IL-23 in the GVL response directed against leukaemia with more aggressive kinetics, similar studies were performed using a Balb-derived A20 leukaemia 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 ALLOGENIC BMT WITHOUT GVHD

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Background: Graft-vs-host disease (GVHD) and lyphepenia 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 (11 Gy) CB6F1 recipient received 5 \(\times 10^6\) T cell depleted BM and 5 \(\times 10^6\) spleenocytes 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 \(\times 10^3\) 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 malignancies receiving myeloablative SCT from matched unrelated donors. Conditioning consisted of cyclophosphamide with Busulphan (n = 61) or total body irradiation (n = 41). Immunosuppression
was cyclosporine with four doses of methotrexate in 97 patients and other treatments in 5. Overall survival at 5 years was 61% and relapse-free survival was 56%. The incidence of acute GVHD grades II-IV was 62% in patients with an LC30 of <0.2 × 10^9/L, 33% if the LC30 was 0.2–1.0 × 10^9/L and 25% in patients with an LC30 >1.0 × 10^9/L (p = 0.008). Transplant related mortality (TRM) was 14% in patients with an LC30 <0.2 × 10^9/L versus 19% (LC30 of 0.2–1.0 × 10^9/L) and 0% (LC30 >1.0 × 10^9/L) (p <0.001). Survival was significantly higher in 17 patients with an LC30 >1.0 × 10^9/L, compared to 67 patients with an LC30 0.2–1.0 × 10^9/L, and 18 patients with <0.2 × 10^9/L (91% vs. 60%, vs. 36% p = 0.02 and 0.001 respectively). When analyzed as a continuous variable in multivariate analysis, an LC30 nadir <0.2 × 10^9/L was associated with a lower incidence of acute GVHD grades II-IV, improved survival, less relapse and higher relapse-free survival. Plasma levels of cytokines were measured in 15 subjects between day 12–32 post-transplant (total 21 samples). Six patients had a low (<0.2 × 10^9/L) and 9 patients a high (>1.0 × 10^9/L) LC30. Plasma IL-15 was lower in patients with high LC30 (median 5.9 pg/ml vs. 35.6 pg/ml, p = 0.05 log rank sum). These results indicate that the LC30 is a robust diagnostic factor for transplant outcome in matched unrelated as well as matched related SCT for myeloid malignancies receiving either BM or PBSC with or without irradiation conditioning. Further research to identify the transplant conditions leading to prompt lymphocyte recovery might lead to global improvements in SCT outcome in unrelated SCT.

28 ADOPITIVE TRANSFER OF NKT CELLS REDUCES GVHD SEVERITY VIA AN IFN-γ AND IL-4 DEPENDENT MECHANISM

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NKT cells, which are CD1d reactive, are thought to play an immunoregulatory role in suppressing dysfunctional immune reactions, including graft-versus-host disease (GVHD). However, we do not know if non-manipulated donor-type NKT can suppress GVHD or how NKT proliferate and migrate following hematopoietic cell transplantation (HCT). We transferred 5.5 × 10^5 highly pure WT NKT cells in C57BL/6 (H-2b) mice into lethally irradiated Balb/c (H-2d) mice receiving NKT had fewer TNFα (CD4: 45% to 27%; CD8 36% to 24%) and IFN-γ levels, which were both significantly elevated only in the plasma of CD4+CD25+FoxP3+ Treg and involved the production of IL-4 and IFN-γ by NKT and a decrease in Tcon which produce TNFα and IL-4 and IFN-γ were high in both NKT treated and untreated groups at day 8 and 10, NKT had also migrated to skin. Total photons emitted from wild-type (WT) C57BL/6 mice, and monitored them by bioluminescence imaging (BLI). By day 4 after transfer, a signal was observed in spleen and lymph node (LN) sites, and between days 7 and 10, NKT had also migrated to skin. Total photons emitted peaked around day 25 after transplantation, followed by a steady decline. To assess the impact of donor-type NKT on GVHD induction by Tcon, we co-transferred various doses of highly pure WT NKT cells in C57BL/6 (H-2b) mice into lethally irradiated Balb/c (H-2d) recipients with 5 × 10^5 T-cell depleted bone marrow (TCD-BM) from wild-type (WT) C57BL/6 mice, and monitored them by bioluminescence imaging (BLI). By day 4 after transfer, a signal was observed in spleen and lymph node (LN) sites, and between days 7 and 10, NKT had also migrated to skin. Total photons emitted peaked around day 25 after transplantation, followed by a steady decline. To assess the impact of donor-type NKT on GVHD induction by Tcon, we co-transferred various doses of highly pure WT NKT at day 0 with 5 × 10^5 TCD-BM, followed by 5 × 10^5 luc + Tcon at day 2. We have found that adoptive transfer of as few as 2.5 × 10^4 NKT can significantly improve survival of mice receiving 5 × 10^5 Tcon. Survival with Tcon only was 20% and for Tcon with NKT was 74%; p = 0.0023. To determine how NKT reduce GVHD, we examined intracellular levels of various cytokines in Tcon with or without 2.5 × 10^5 NKT, following HCT. At 8 days after HCT, mice receiving NKT had fewer Tnfα-positive cells from LNs (CD4: 45% to 27%; CD8 36% to 24%); by day 11, however, Tnfα levels between groups were equivalent. IFN-γ levels, which were high in both NKT treated and untreated groups at day 8 (85%-95%), decreased significantly in NKT treated mice by day 11 (CD4: 40%; CD8: 43%), but were abundant in Tcon only mice (CD4: 78%; CD8: 80%) (p = 0.0001). NKT from both IL-4-/- and IFN-γ-/- mice were less effective at suppressing GVHD than WT NKT, implicating these cytokines in the suppressive mechanism. Finally, we found that NKT do not have a major impact on the graft-versus-tumor effect of Tcon against a luc +BCL-1 tumor. These studies indicate that NKT persist in vivo upon adoptive transfer and suppress GVHD, even at extremely low cell numbers, which is important given the relative paucity of this cell population. The mechanisms of GVHD suppression was also found in those of CD4+CD25+FoxP3+ Treg and involved the production of IL-4 and IFN-γ by NKT and a decrease in Tcon which produce pro-inflammatory cytokines.

29 CEACAM1 REGULATES EXPERIMENTAL GRAFT-VERSUS-HOST-DISEASE

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Carinoembryonic antigen associated cell adhesion molecule (Ceacam1) is a type I transmembrane glycoprotein that regulates numerous processes including bacterial colonization of the gastrointestinal lumen and leukocyte function. Ceacam1 is expressed on gut epithelium and activated T cells, particularly in the intestines. We found that T cells from Ceacam1-/- mice had elevated phosphorilation of STAT3. Upon stimulation with anti-CD3-CD28, IL-2, IL-4, IL-6, or IL-12 in vitro, Ceacam1-/- T cells showed hyperphosphorylation of corresponding canonical STAT proteins, indicating that Ceacam1 can regulate the sensitivity of T cells to cytokines and TCR stimulation. Ceacam1-/- T cells also had defective anergy induction as measured by IL-2 secretion. This was associated with decreased cell death and increased apoptosis) and hypophosphorylation of STAT1 upon IFNy treatment. We assessed Ceacam1 regulation of T cells in vivo during GVHD. Ceacam1-/- T cells caused increased mortality and large intestinal GVHD, had more profound activation (CD25, CD62L) and expression of integrin α4β7 and demonstrated selective infiltration of the intestines. By contrast, Ceacam1-overexpressing T cells caused significantly less GVHD mortality and damage to all target organs, which correlated with decreased proliferation and organ infiltration. We also studied Ceacam1 in recipients of allogeneic bone marrow transplantation (allo-BMT), and found that compared to wildtype (WT) recipients, Ceacam1-/- recipients with GVHD showed accelerated mortality and increased damage to the large intestines and thymus. Donor alloactivated CD4 T cells in Ceacam1-/- allo-BMT recipients had increased activation (CD25), and trafficked preferentially to the mesenteric lymph nodes, small and large bowel, but had decreased accumulation in the liver and peripheral lymph nodes (PLN). Finally, Ceacam1-/- mice were more sensitive to radiation injury as demonstrated by accelerated kinetics of mortality and decreased numbers of surviving or regenerating small intestinal crypts. We conclude that Ceacam1 is an important regulator of alloreactivity during GVHD through its effects on T cell (allo)activation, proliferation, trafficking, cytokine sensitivity, and anergy. Moreover, Ceacam1 expression on gut epithelium regulates sensitivity to radiation injury, T cell alloreactivity, and intestinal GVHD.

30 ELAFIN IS A BIOMARKER OF GRAFT VERSUS HOST DISEASE OF THE SKIN

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There are no plasma biomarkers specific to any of the three target organs of acute graft versus host disease (GVHD): skin, GI tract and liver. We sought to identify a biomarker for skin GVHD in an initial discovery step using an intact proteome analysis system. We compared plasma pooled from ten patients with skin GVHD only (gGVHD) to ten patients without GVHD (–GVHD) to ten patients with GI tract GVHD only (gGVHd). Of four candidate proteins that were both significantly elevated only in the plasma of gGVHD and that could be measured by ELISA, ELAFIN, an epidermal proteinase inhibitor that is induced by TNF-α and found in inflamed epidermis. We measured levels of elafin