IL-2. IL-2-expanded CB NK cells formed increased immune synapses with K562 tumor cells (mean 65%; 60–71%) and primary human AML blasts (mean 48%, range 39–55%), comparable to the levels generated with peripheral blood NK cells. Additionally, we demonstrated that *ex vivo* expanded CB NK cells could efficiently kill human AML engrafted in a NOD/scid/IL-2Rg-null mouse model. A mean 50% (37–66%) reduction in AML blasts was observed in comparison to control groups (mean 3%;range 2.4–4.7%) by 6 weeks post NK infusion ($p<0.05$). Our results suggest that *ex vivo* expansion of CB NK cells is a feasible and effective strategy for the treatment of AML.

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CHARACTERIZATION OF CTL CLONES ISOLATED FROM BONE MARROW TRANSPLANT RECIPIENT WITH HLA-CW-MISMATCHED DONOR

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HLA-Cw disparity in a donor increases the risk of severe acute GVHD after BMT. However, HLA-Cw-specific cytotoxic T lymphocytes (CTLs) generated in post-transplant recipients who develop acute GVHD have not been characterized in detail. Here, we characterized CTL clones isolated from a recipient at the onset of grade II acute GVHD who was transplanted from an HLA-A, B and DRB1-matched, Cw-mismatched (recipient, Cw*0303/Cw*0702; donor, Cw*0801/Cw*0702) unrelated donor. Seven CTLs were isolated. Four CTLs were CD8-positive, one was CD4-positive, and two were CD4/CD8-double positive. Three of those including a CD8-positive CTL, a CD4-positive CTL and a CD4/CD8-double positive CTL had exactly the same nucleotide sequences in the CDR3 region of their T cell receptors, suggesting these three CTLs with variable phenotypes originated from a single clone. All CTLs lysed Epstein Barr virus-transformed lymphoblastoid cell (B-LCL) from the recipient and donor B-LCL transfected with the HLA-Cw*0303 cDNA construct, but failed to lyse B-LCL from the donor. COS cells transfected with HLA-Cw*0303 cDNA construct stimulated IFNγ production by all CTLs. Thus, all isolated CTLs recognized HLA-Cw*0303 molecule as an alloantigen. COS cells transfected with Cw*0303 mutants in which amino acids constituting peptide-binding pockets (aa position: 114, 116, 152, or 163) were substituted with Cw*0801-amino acids could not stimulate IFNγ production by any CTLs, whereas COS cells transfected with Cw*0303 mutants bearing Cw*0801-amino acids outside the positions constituting peptide-binding pockets stimulated all CTLs as well as the wild-type Cw*0303 construct. These data indicated that peptides bound to Cw*0303 molecules influence the allorecognition of Cw*0303-specific CTLs. Taken together, our findings suggest, although the immunogenicity of HLA-Cw antigens is considered to be low as a consequence of their low level of cell surface expression, HLA-Cw-specific CTL clones with a variable phenotype can naturally arise in post-transplant recipients and cause acute GVHD through recognition of the peptides bound to Cw molecules. This is consistent with a recent statistical study showing a significant association between some specific amino acid substitutions at positions constituting peptide-binding pockets of the HLA-Cw molecule and the occurrence of severe acute GVHD after unrelated BMT.

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A ROLE FOR NF-KB INDUCING KINASE (NIK) IN GRAFT VERSUS HOST REACTIONS

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NF-KB is a family of transcription factors with an important role in immune and inflammatory responses, and represents a target for the treatment of immune diseases. NF-KB inducing kinase (NIK) is required for the activation of the NF-KB noncanonical (or alternative) pathway. We have studied the role of NIK in graft versus host disease (GVHD) in humans and in a murine model of GVHD. Human T lymphocytes activated in vitro (CD3 + CD28 antibodies, and/or TNF-α) showed an initial downregulation of NIK, followed by an increase above basal levels, during the first 48 hours. On the other hand, circulating T lymphocytes from patients with acute GVHD (aGVHD) expressed NIK while T cells from healthy donors did not, and T lymphocytes infiltrating the dermis of pathological lesions of those patients expressed high level of NIK. We next explored the effects of suppressing NIK in a murine model of GVHD in mice: $H2D^b$ T lymphocytes transplanted into fully mismatched $H2D^d$ recipients. Donor mice were either *aly/aly* (NIK deficient) or C57BL/6 (control), and recipient mice were Balb/c. Mice recipient of BL6 cells developed a severe form of aGVHD and died in the third week after transplant. On the contrary, mice transplanted with aly T lymphocytes survived for 3 months and did not develop GVHD Histopathological analysis of skin, gut and liver of these surviving mice showed no sign of GVHD. We next studied the fate of the infused T lymphocytes in the early days postransplant, in order to ascertain the cause of the difference in GVHD survival. We found that aly and BL6 T lymphocytes had the same capacity for homing into the spleens, but the clonal expansion of aly T lymphocytes was significantly impaired at day 5. Analysis of in vivo T cell divisions showed the same kinetics for aly and BL6 T lymphocytes at 1, 3 and 5 days after transplant. Therefore, aly T cells divided *in vivo* but the process was not productive. Finally, the proportions of apoptotic aly T lymphocytes were higher than those of BL6, at days 1 and 3, but not at day 5. We also detected lower amounts of Th1 cytokines levels in the serum of mice receiving aly *vs.* BL6 T cells, 5 days after transplant. In summary, NIK-deficient T lymphocytes had a normal proliferative capacity but an increased apoptotic rate early after transplant compared to controls, and were unable of mounting a successful GVH reaction in the fully mismatched allogeneic setting.

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PLASMA CYTOKINE LEVELS DISTINGUISH CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) SYMPTOM PROFILE GROUPS

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Background: Understanding the biologic milieu associated with differing symptom profiles in allogeneic HSCT survivors with cGVHD has the potential to clarify the pathogenesis of cGVHD activity and tissue damage, and may direct the development of new strategies to ameliorate symptoms and improve quality of life.

Objective: To determine if differing cGVHD symptom profiles are associated with unique patterns of pro-inflammatory cytokine expression and markers of systemic immune activation.

Methods: Plasma levels of sBAFF, IL-1β, IL-1 receptor antagonist, IL-6, soluble IL-6 receptor, soluble TNF-receptor II, MCP-1, and MIG were assayed by sandwich ELISA. Lymphocyte subset quantities were measured. Concurrently, the Lee Chronic GVHD Symptom Scale, a self-report measure of cGVHD symptoms, and clinical data were obtained. Data from 79 patients were analyzed using descriptive statistics, latent profile analysis, and multinomial logistic regression.

Results: Three distinct symptom subgroups were identified through latent profile analysis: Group 1 (low on all symptoms), Group 2 (prominent oral, upper GI, and weight loss symptoms); and Group 3 (eye, muscle/joint, fatigue and mood symptoms). Multinomial logistic regression, controlling for age, length of time since transplant, cGVHD severity, intensity of immunosuppression and post-transplant lymphocyte reconstitution, revealed that IL-6, TNF-RII, MCP-1 and sBAFF levels significantly distinguished the three symptom subgroups.

Odds Ratios and 95% Confidence Intervals for the Multinomial Logistic Regression of Symptom Latent Class Membership on Cytokines † Controlling for Age, Time Since Transplant, cGVHD Severity, Intensity of Current Immunosuppression and Absolute Lymphocyte Counts †

	Symptom Profile 1 (low on all symptoms) vs. Symptom Profile 2 (prominent oral/ GI symptoms) OR (95% CI)	Symptom Profile 1 (low on all symptoms) vs. Symptom Profile 3 (prominent eye, muscle/joint, fatigue and mood symptoms) OR (95% CI)	Symptom Profile 2 (prominent oral/ GI symptoms) vs. Symptom Profile 3 (prominent eye, muscle/joint, fatigue and mood symptoms) OR (95% CI)
IL-1 β	1.59 (0.30–8.45)	0.92 (0.32–2.60)	0.58 (0.10–3.32)
IL-6	0.27 (0.09–0.79)*	1.15 (0.52–2.52)	4.26 (1.32–13.82)*
IL-1 RA	0.69 (0.22–2.14)	0.55 (0.29–1.07)	0.81 (0.26–2.48)
sIL-6R	0.50 (0.12–2.13)	1.78 (0.54–5.90)	3.53 (0.83–15.13)
TNF-RII	0.29 (0.04–2.42)	0.28 (0.08–0.99)*	0.95 (0.12–7.59)
MCP-1	8.70 (1.46–51.66)*	0.40 (0.11–1.40)	0.05 (0.01–0.36)**
MIG	1.33 (0.73–2.44)	1.02 (0.63–1.65)	0.77 (0.42–1.39)
sBAFF	0.40 (0.13–1.30)	1.86 (0.88–3.93)	4.61 (1.39–15.31)**

† values were log normal transformed prior to analysis
**or *$p < .01$ or $p < .05$, respectively, multinomial logistic regression.

Higher IL-6 levels significantly differentiated participants in Group 2 from those in Groups 1 and 3. Group 3 had higher TNF-RII compared to Group 1. Those with higher sBAFF levels were significantly more likely to be in Group 2, while those with higher MCP-1 levels were significantly more likely to be in Group 3.

Conclusion: Allogeneic HSCT survivors with differing cGVHD symptom profiles were distinguished by significantly different levels of IL-6, TNF-RII, MCP-1 and sBAFF. These data validate this new symptom grouping system based on the Lee Chronic GVHD Symptom Scale as a measure of cGVHD disease burden. IL-6, TNF-RII, MCP-1 and sBAFF appear to be important biomarkers that reflect specific cGVHD manifestations and merit further study.

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DONOR DENDRITIC CELLS INITIATE COUNTER-REGULATORY IMMUNE RESPONSES AND GVL EFFECTS IN ALLLOGENEIC BMT

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Based on a clinical association of donor plasmacytoid dendritic cell (DC) content with leukemia relapses after allo BMT (Waller, Blood 2001), we previously reported that donor $CD11b^-$ DC enhanced IFN-γ synthesis and GvL activity of donor T-cells, while $CD11b^+$ DC resulted in increased IL-10 production and decreased GvL function by donor T-cells in allo BMT mouse models (Li, Blood 2007). In this study, we tested mechanisms whereby donor DC in the graft modulate donor T-cell activation in a MHC-mismatched (C57BL/6→B10.BR) allo BMT. Recipients received 11 Gy irradiation followed by tail vein injection of purified donor HSC, DC subsets and T-cells two days later. Allografts consisted of 5×10^4 FACS-purified donor BM $CD11b^-$ DC or $CD11b^+$ DC plus 3×10^3 $c\text{-}kit^+sca\text{-}1^+lineage^-$ HSC in combination with either 3×10^5 T-cells or no additional T-cells. In vivo donor-derived T-cell proliferation was assessed by CFSE staining. Serum and intracellular cytokines (Th1: IL-12, IFN-γ, IL-2, and TNF-α and Th2: IL-4, IL-5, and IL-10) were tested by ELISA and flow cytometry. Costimulatory molecule expression(CD40, CD80, ICOSL, PD-L1 and PD-L2) was measured by flow cytometry following recovery of GFP^+ donor DC on day_{+10} post-transplant. We found that donor CD8 T-cells had higher levels of Ki-67 expression and proliferation than donor CD4 T-cells 3 days post-transplant following transplantation with $CD11b^-$ DC compared with $CD11b^+$ DC, and without donor DC ($p < 0.001$). Both $CD11b^-$ DC and $CD11b^+$ DC had similar level of expression of CD40, CD80, and ICOSL in BM, in lymph nodes and in spleen at 10 days post-transplant, but $CD11b^+$ donor DC recovered from BM had much higher levels of PD-L1 than $CD11b^-$ DC, while $CD11b^-$ DC in all tissues had higher levels of PD-L2. Donor $CD11b^-$ DC enhanced Th1 cytokine production of donor T-cells, while donor $CD11b^+$ DC elevated Th2 cytokine production compared with T-cell alone. In conclusion: Donor $CD11b^-$ DC enhanced donor T-cell proliferation, especially CD8 T-cells compared with donor $CD11b^+$ DC or recipients of T-cells alone. Homing of donor DC to lymphoid organs and expression of costimulatory molecules on donor $CD11b^-$ and $CD11b^+$ DC subsets *in vivo* were similar, suggesting that the differences observed in donor T-cell activation and proliferation in distinct donor DC subset is likely due to local production of cytokines by donor DC. The limited GvHD seen with T-cells injected with $CD11b^-$ DC may be due to differential expression of PD-L2 compared with $CD11b^+$ DC.

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C-FLIP EXPRESSION DETERMINES LOW SENSITIVITY OF TYPE 17 T HELPER CELLS TO ACTIVATION-INDUCED CELL DEATH

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T cell apoptosis induced by repeated TCR stimulation, known as activation-induced cell death (AICD), plays an important role in T cell tolerance. Pro-inflammatory, IL-17-producing $CD4^+$ T cells (Th17 cells) have been recently identified as a unique T helper subset. Preliminary work from our lab indicated that Th17 cells participate in the pathogenesis of acute graft-versus-host disease (GVHD). In this study, we compared the susceptibility of polarized Th1 and Th17 effectors to AICD *in vitro* and *in vivo*. We found that Th17 effectors were significantly less susceptible to AICD upon TCR restimulation compared to Th1 effectors. It was further confirmed by Th17 effectors generated from *il17f/rfp* knock-in mice, RFP^+ (Th17) cells were indeed more resistant to AICD than RFP^- (non-Th17) cells. Resistance of Th17 cells to AICD was also observed when Th17 cells were co-cultured with Th1 cells *in vitro* or co-injected with Th1 cells *in vivo* in allogeneic recipients. To explore the molecular mechanism of AICD resistance in Th17 cells, we found that Th17 cells were defective in expression of FasL and in activation of caspases, but highly expressed anti-apoptotic protein c-FLIP as compared to Th1 cells. Given that Th17 cells were resistant to AICD when co-cultured with Th1 cells that express high levels of FasL, we hypothesized that Fas-signaling was impaired on Th17 effectors. After knocking down c-FLIP with its specific siRNA, Th17 cells upregulated FasL and underwent rapid apoptosis upon TCR restimulation, indicating that Th17 cells are resistant to AICD likely because the high level of c-FLIP prevents Fas-mediated apoptosis. These results suggest the resistance of Th17 cells to AICD as additional mechanism that contributes to the high pathogenicity of Th17 cells in autoimmune diseases and GVHD. Our finding also strengthens the rationale to use tumor-specific Th17 cells for adoptive cell therapy in cancer.

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MESENCHYMAL STEM CELL INFUSION AS PREVENTION FOR GRAFT REJECTION AND GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING FROM HLA-MISMATCHED DONORS: A PILOT STUDY

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Background: Allogeneic hematopoietic cell transplantation (HCT) following nonmyeloablative conditioning has been an effective treatment for many pts with hematological malignancies who have a HLA-matched related or unrelated donor. However, results of nonmyeloablative HCT in pts with HLA-mismatched donors have been disappointing due to high incidence of graft rejection and severe acute GVHD. Recent studies have suggested that infusion of mesynchymal stem cells (MSC) the day of HCT might promote engraftment and prevent acute GVHD after myeloablative allogeneic HCT. This prompted us to investigate whether MSC infusion a few hours before HCT could allow non-myeloablative HCT from HLA-mismatched donors to be performed safely (i.e. with a 100-day incidence of nonrelapse mortality <35%).