lung GVHD is currently still on etanercept and stable off steroids. Five patients are still alive currently. Three patients had SD and one patient had PD on etanercept. Causes of death included cGVHD (n=2), disease progression (n=1) and unknown cause (n=1). Conclusion: In this preliminary evaluation, etanercept was well tolerated and had activity in patients with cGVHD of the skin, and potentially in some cases with visceral involvement and failing corticosteroids. Further dosing and efficacy studies earlier in the treatment of patients with cGVHD are warranted.

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ESTABLISHMENT OF A CHROMIDIC NOD-SCID/IL2Rγnull TRANSPLANTATION-MODEL TO EVALUATE GRAFT-VS-HOST AND GRAFT-VS-LEUKEMIA IMMUNE RESPONSES OF EX VIVO MODIFIED HUMAN T LYMPHOCYTE GRANTS
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Donor lymphocyte graft engineering to abrogate graft-vs-host (GVH) reactivity while improving graft-vs-leukemia (GVL) immunity is of particular interest in allogeneic hematopoietic stem cell transplantation. We have recently described a protocol to achieve short-term expansion of donor-derived leukemia-reactive T cells ex vivo followed by CD137-mediated selective depletion of alloreactivity (SAD) to major and ubiquitously expressed minor histocompatibility antigens using allogeneic fibroblasts. For evaluating GVL and GVL immune responses of modified donor T cells in vivo we have now used NOD-scid IL2Rγnull mice to establish a chimeric transplantation model. GVL reactivity after SAD was examined by subcutaneously implanting skin substitutes composed of a collagen-based matrix which contained 6x10^5 human primary fibroblasts derived from the same pool previously used for SAD. Upon implantation, substitutes remained viable, induced angiogenesis and could be dissolved to cell suspensions for further analyses when removed 3 weeks after implantation. Following intravenous injection of 1.5x10^7 preactivated untreated allogeneic human CD34+ T cells per mouse, 7-10% of T cells migrated into the skin substitutes explanted 14 days post adoptive transfer. Such an enrichment of T cells was not observed when the same number of CD137-allodepleted T cells were injected. Since xenogeneic GVL reactivity was observed depending on the amount of T cells used current studies investigate whether residual murine antigen-presenting cells initiate xeno-reactivity and address its impact on the migration of T cells into the skin substitutes. To study GVL immunity we further transplanted different amounts of acute myeloid leukemia (AML) cells derived from primary AML patients (M4 and M5 subtype) into NOD-scid IL2Rγnull mice. Eight weeks after injection 61% and 17% of isolated spleen and bone marrow cells, respectively, stained positive for a shared HLA-class-I epitope and also expressed CD34 as measured by flow cytometry. Currently, studies are in progress to examine immune responses to AML by adoptive transfer of human AML-stimulated allodepleted T cell lines devoid of alloreactivity to AML HLA-matched fibroblasts. In summary, our NOD-scid/IL2Rγnull transplantation model may be a valuable tool for evaluating GVL and GVL immunity in vivo following modifications of donor T lymphocytes grafts in vitro.

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ALLOANTIGENS EXPRESSION ON HOST NON-HEMATOPOIETIC CELLS LEADS TO DONOR T CELL EXHAUSTION AND REDUCES GVL EFFECTS
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We previously showed that alloantigen expression on host non-hematopoietic cells impaired graft-versus-leukemia (GVL) effects after allogeneic hematopoietic stem cell transplantation using chimeric mice expressing alloantigens on hematopoietic cells alone. C3.Sw (H-2b) mice were lethally irradiated and injected with T cell depleted bone marrow cells (TCD-BM) from multiple minor histocompatibility antigens (mHAs) disparate B6 (H-2b) donors. These chimeric mice (Bc chimeras), expressing B6-derived mHAs only on hematopoietic cells, were lethally irradiated and transplanted with TCD-BM and C3B T cells from C3.Sw donors together with B6-derived leukemia cells, EL-4. In controls we used B6→B6 chimeras (Bb chimeras), expressing B6-derived mHAs on both hematopoietic and non-hematopoietic cells, as recipients. BB chimeras transplanted with TCD-BM + CDS B T cells from C3.Sw exhibited a significant GVL effect but died from leukemia significantly earlier than BC chimeras. To further confirm that alloantigen expression on non-hematopoietic cells impairs GVL activity, similar experiments were performed using B6→B6-82m deficient chimeras (B- chimeras), lacking functional MHC class I molecules on non-hematopoietic cells. Leukemia mortality was significantly reduced in B- chimeras compared to BB chimeras, thus confirming that alloantigen expression on host non-hematopoietic cells impairs GVL effects. To elucidate the mechanism of the reduced GVL effects in BB chimeras, T cells were isolated from lymph nodes and spleen of chimeras after BMT. Numbers of donor CDS+ T cells in lymph nodes and spleens of BB recipients decreased much earlier than BC chimeras in association with an enhanced apoptosis. CTL activities against EL-4 significantly reduced in BB recipients compared to BC and B- chimeras. PD-1 expressions on CD8+ T cells from BB chimeras were significantly enhanced compared to those from BC chimeras. These results suggest that alloantigen expression on host non-hematopoietic cells leads to exhaustion and dysfunction of donor T cells and impairment of GVL effects.

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THE ROLE OF SNPS WITHIN RECEPTORS OF INNATE IMMUNITY IN OUTCOME FOLLOWING ALLOGENEIC STEM CELL TRANSPANTATION: SYNERGISM BETWEEN TLR5-STOP AND NOD2/CARD15?
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Our group recently reported an association of single nucleotide polymorphisms (SNPs) within NOD2/CARD15, an important intracytoplasmatic receptor of the bacterial ligand myramyl-dipeptide, with severe GVHD, treatment related mortality (TRM) and outcome following allogeneic stem cell transplantation (SCT). As these studies suggested a major role of dysregulated epithelial inflammation, we now tested the role of SNPs within further receptors involved in cellular sensing of bacterial ligands. SNPs reported for TLR2, TLR3, TLR4, TLR5 and TLR9 were assessed in recipient (R) and donor (D) DNA from 259 HLA-identical sibling and 294 matched unrelated donor transplants. Acute GVHD grade II/IV, 1-yr- and overall TRM as well as overall survival were correlated with results of SNP typing, and the influence of major clinical risk factors as well as previously assessed presence or absence of NOD2/CARD15 SNPs was investigated with univariate and multivariate analyses.

Whereas major SNPs within TLR2, 3, 4 and 9 failed to show clear associations, the presence of a TLR5-stop codon in the recipient (n=58) showed a trend for higher severe GVHD (22%
with a 28% increase in TRM (51% vs 33%, p = 0.002) as well as overall TRM, which translated into decreased overall survival (51% vs 33%, p = 0.002). The effect was strongest in the subgroup of younger and early stage pts receiving HLA-identical sibling SCT; in addition, combined analysis of TLR5-stop and NOD2/CARD15 SNPs suggested a potential synergism, as 1 yr TRM was 21% in the presence of both SNPs, 52 and 33% in the presence of either TLR5-stop or NOD2/CARD15 and 66% in the presence of both. In multivariate Cox regression analysis of risk factors for 1 yr TRM, older recipient age (HR 1.5, p = 0.004), female donor in a male recipient (HR 1.7, p = 0.01) and presence of TLR5-stop (HR 1.6, p = 0.05) and NOD2/CARD15 (HR 1.8, p = 0.004) in the recipient were confirmed as independent risk factors.

As both TLR5-stop and NOD2/CARD15 SNPs have been associated with functional defects, our observations further support the concept of a major role of altered innate immune responses in the pathophysiology of GvHD associated complications following allogeneic SCT.

Poster Session II

300 IMMATURE DENDRITIC CELLS DOWNREGULATE GRAFT VERSUS HOST REACTIONS IN THE HUMAN SKIN EXPLANT MODEL AFTER CO-CULTURE WITH IN VITRO PUVA TREATED LYMPHOCYTES

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Poster Session II

301 SELECTIVE EXPANSION OF HUMAN REGULATORY (CD4+CD25+CD127lowFOXP3+) CELLS TO HIGH PURITY BY INHIBITING EXPANSION OF CD4+CD25+CD127highFOXP3+ CONVENTIONAL T CELLS WITH RAPAMYCIN

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CD4+CD25+FOXP3+ natural occurring T regulatory (Treg) cells possess great therapeutic potential as adoptive cellular therapy for controlling acute GVHD. However, their clinical application is limited by the difficulty of obtaining sufficient numbers of CD4+CD25+FOXP3+ cells. This study explored a strategy to expand human Treg in high purity and high numbers. By comparing expression of cell surface molecules on sorted CD4+CD25+ vs. CD4+CD25- cells using mAb microarray, we identified CD127 was highly expressed in CD4+CD25+ and CD4+CD25- cells in human PBMC. Incorporating this new discovery, CD4+CD25+ cells were isolated by CD25+ selection from PBMC, sorted into CD4+CD25+CD127low and CD4+CD25+CD127high cells. FOXP3 were positive in 94% of CD4+CD25+CD127low cells, and 5% in CD4+CD25+CD127high cells. The sorted cells were expanded with and without rapamycin in X-vivo medium containing IL-2 and anti-CD3/CD28 beads for a total of 21 days. In the absence of rapamycin, CD4+CD25+CD127low cells expanded expanded 156 and 1560 folds, yet only 30% and 20% of expanded cells were positive for FOXP3 at day 14 and 21 respectively. In the presence of rapamycin CD4+CD25+CD127low cells remain FOXP3+ in 96%, 60% and 56% at days 7, 14 and 21. However, the number of cells increased only 19, 36, and 21 folds at days 7, 14 and 21 respectively. To overcome the insufficient expansion of CD4+CD25+CD127low cells in the presence of rapamycin, anti-CD3 mAb (OKT3) or anti-CD3/CD28 beads was added to the media at day 7 after the initial beads were removed and cultured until day 21. At day 14, 66% of the cells were FOXP3+ positive with rapamycin and anti-CD3 and 80% for cells with rapamycin and anti-CD3/CD28 beads. The numbers of cells were expanded: 13 folds with rapamycin and anti-CD3, and 238 folds with rapamycin and anti-CD3/CD28 beads. At day 21, the numbers of cells were expanded: 34 folds with rapamycin and anti-CD3, and 2865 folds with rapamycin and anti-CD3/CD28 beads. In contrast, CD4+CD25+CD127low cells expanded in the same condition as controls had much lower percentages of anti-CD3+ cells. In summary, in the presence of rapamycin, CD4+CD25+CD127low+FOXP3+ cells were preferentially expanded with IL-2 and anti-CD3/CD28 beads. Continued stimulation from anti-CD3/CD28 beads enhanced the expansion of CD4+CD25+CD127low+FOXP3+ cells. The suppressive function was positively correlated with the percentage of FOXP3+ cells in the MLR culture.

302 TREATMENT OF SEVERE OCULAR SURFACE DISEASE FROM OCULAR CHRONIC GRAFT-VERSUS-HOST DISEASE WITH A SCERAL LEN PROSTHETIC DEVICE

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Purpose: The fluid-ventilated, gas-permeable scleral lens prosthetic device is an innovative treatment for ocular surface disease. Retrospective studies of patients with severe dry eye fitted with these lenses report mitigation of symptoms, improvement in function and quality of life, and healing of persistent epithelial defects of the corneal surface. This prospective study is designed to measure the impact of this device on visual function in patients with ocular chronic graft-versus-host disease (cGVHD) unresponsive to conventional therapy.

Methods: All patients referred to this institution for scleral lens consultation from January through June 2006 were administered the VFQ-25 at entry, and again 6 months later. The National Eye Institute sponsored the development of the VFQ-25 as a standardized, validated survey instrument to measure the dimensions of self-reported vision-targeted health status in persons who have