alloreactive responses, mixed lymphocyte reactions (MLR) were performed (n = 6). High-dose, activated cyclophosphamide (mafosfamide) given 3 days after the start of MLR caused increases in Fr I-III at 7 days (Fr II, $p<0.0001$; Fr III, $p = 0.0002$), while cyclosporine-treated cells had similar to lower levels of these fractions compared with controls. The addition of mafosfamide at day 3 to cyclosporine-cultured cells in MLR caused similar increases in Fr I and III as seen with mafosfamide alone, but the Fr II increase was no longer significant. In summary, T cell reconstitution in patients treated with PT/Cy differs from that seen with CNI-based GVHD prophylaxis. For patients treated with PT/Cy who still develop aGVHD, relative expansion of Tregs may be a critical compensatory mechanism accounting for the remarkably low incidence of chronic GVHD seen in these patients.

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IMMUNE INSUFFICIENCY AFTER EXPERIMENTAL TRANSPLANTATION IS DUE TO DEFECTIVE ANTIGEN PRESENTATION WITHIN DENDRITIC CELL SUBSETS

Markey, K.A.[1], Koyama, M.[1], Kuns, R.D.[1], Lineburg, K.E.[1], Wilson, Y.A.[1], Olver, S.D.[1], Don, A.L.J.[1], Varelias, A.[1], Robb, R.J.[1], Cheong, M.[1], Engwerda, C.R.[2], Steptoe, R.J.[3], Ramshaw, H.S.[4], Lopez, A.F.[4], Lew, A.M.[5], Villadangos, J.A.[5], Hill, G.R.[1], MacDonald, K.P.A.[1,1] Queensland Institute of Medical Research, Brisbane, Queensland, Australia; [2] Queensland Institute of Medical Research, Brisbane, Queensland, Australia; [3] University of Queensland Diamantina Institute, Brisbane, Queensland, Australia; [4] Centre for Cancer Biology, South Australian Pathology, Adelaide, South Australia, Australia; [5] Walter and Eliza Hall Institute, Melbourne, Victoria, Australia

Alloreactivity after transplantation is associated with profound immune suppression and consequent opportunistic infection results in high morbidity and mortality. This immune suppression is most dramatic during graft–versus–host disease (GVHD) after bone marrow transplantation (BMT) where an inflammatory cytokine storm dominates. We used the B6 ($I\text{-}A^b/I\text{-}E^{-/-}$) → BALB/c ($I\text{-}A^d/I\text{-}E^d$) BMT model to track antigen specific responses induced by donor APC. Bone marrow grafts were either T cell depleted (non-GVHD) or replete (GVHD) and derived from wild-type or CD11c.OVA transgenic (Tg) donors. We determined the extent of antigen presentation by quantifying CFSE dilution of adoptively transferred TEa Tg T cells (which respond to host $I\text{-}E^d$ presented in donor $I\text{-}A^b$) and OT-II Tg T cells (which respond to OVA-derived peptide). GVHD induced a marked defect in antigen presentation of both alloantigen and nominal antigens. By utilizing CD11c.DTR Tg grafts and DC-depletion with diphtheria toxin we demonstrated that conventional DC (cDC) were the critical donor cell presenting alloantigen during GVHD. Surprisingly, number, phenotype and phagocytic/proteolytic function of these cells were all equivalent in the GVHD and non-GVHD settings. However, cDC from mice with GVHD failed to present OVA Ag within MHC II to OT-II T cells, confirming a DC specific defect during GVHD. In contrast, both classical and cross presentation within MHC class I remained intact. Analysis of cDC subsets revealed that the $CD8^{neg}$ donor cDC were the critical cell population for class-II mediated Ag presentation and that this specific sub-population was defective during GVHD. This defect is two-fold, and is caused by both a functional impairment in class II presentation in the $CD4^{neg}CD8^{neg}$ cDC population, and a concurrent failure of development in the $CD4^{+}CD8^{neg}$ population. Importantly, the functional defect in antigen processing within MHC class II can be reversed by TNF inhibition or the adoptive transfer of donor cDC generated in the absence of inflammation.

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TOLERANCE ASSOCIATED GENE EXPRESSION FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

Pidala, J.[1], Bloom, G.[2], Eschrich, S.[2], Enkemann, S.[3], Anasetti, C.[1,1] Moffitt Cancer Center, Tampa, FL; [2] Moffitt Cancer Center; [3] Moffitt Cancer Center

Background: Elucidation of immune tolerance-associated biomarkers may facilitate personalized management of immune suppression (IS) following allogeneic hematopoietic cell transplantation (HCT).
Methods: We have performed an analysis of differential gene expression between tolerant, non-tolerant, and healthy control subjects. Tolerant cases (n = 17) were HCT recipients off IS for at least 6 months without any manifestations of acute or chronic graft vs. host disease (GVHD). Non-tolerant comparators (n = 16) were matched for age (+/- 5 years) and time from date of HCT (+/- 6 months), but failed to discontinue IS due to GVHD. Published gene expression data from healthy volunteer subjects (n = 9) served as control. Clinical data gathered on all tolerant (TOL) and non-Tolerant (non-TOL) cases included patient demographic and HCT information, comprehensive review of acute and chronic GVHD activity, and history of IS drug therapy. From freshly obtained peripheral blood samples, two analyses were performed: First, from total peripheral blood mononuclear cells (PBMC), cell populations were characterized by surface phenotype with flow cytometry. Second, RNA was extracted from fresh PBMC to serve as the mRNA source for microarray analysis using the Affymetrix Human U133 plus 2.0 array.
Results: Demographic and transplantation variables did not significantly differ between groups. We did not detect significant differences in cell composition (αβ T cells, γδ T cells, regulatory T cells, NKT cells, NK cells, B cells, monocytes, type 1 and type 2 dendritic cells) between TOL and non-TOL groups. We first conducted a two group (TOL vs. non-TOL) comparison of gene expression. SAM analysis with 300 permutations, ≥ 1.5-fold change, and 10% false discovery rate identified 231 genes up- and 412 down-regulated in Tol vs. non-Tol groups. Pathway analysis (MetaCore by GeneGo) mapped involved genes to immune response pathways involving T cell (TCR receptor signaling, NF-AT) and NK cell (DAP12, NKG2D, CD16) signaling. Construction of a 3-way (TOL vs. non-TOL vs. control) classifier resulted in a weighted accuracy of 88% for leave 10% out cross-validation.
Conclusion: These data demonstrate differential gene expression among tolerant HCT recipients distinct from both non-tolerant and control subjects, and support a classifier that can accurately identify the tolerant phenotype. Confirmation of these results will require a prospective trial of IS discontinuation.

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THYMIC FUNCTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS DEPENDENT ON GRAFT SOURCE AND PREDICTIVE OF LONG TERM SURVIVAL

Sairafi, D.[1,2], Mattsson, J.[1,2], Uhlin, M.[1,2], Uzunel, M.[2] [1] Karolinska University Hospital, Stockholm, Sweden; [2] Karolinska Institutet, Stockholm, Sweden

Background: T-cell function is defective after allogeneic stem cell transplantation (ASCT). This has a significant clinical impact by increasing patients' susceptibility to infections, thus reducing survival. One way to assess thymic output of T-cells is by measuring the level of T-cell receptor excision circles (TRECs).
Methods: This retrospective study involved 210 patients with hematological malignancies. TREC levels in separated T-cells were measured at 6 time-points during the first 24-months after ASCT, using quantitative real-time PCR.
Results: The use of bone marrow grafts rather than peripheral blood stem cell grafts showed a significant correlation with higher TREC levels >6 months after ASCT ($p<0.001$). Treatment with anti-thymocyte globulin was correlated with lower TREC levels ≤6 months post-ASCT ($p<0.001$). The group of patients with TREC levels above median 3 months post-ASCT had a superior overall survival; 80% vs. 56% ($p = 0.002$), and lower transplantation-related mortality; 7% vs. 21% ($p = 0.01$).
Conclusion: These results give further evidence that thymic function has an important role in the reconstitution of the T-cell pool after ASCT. We have also shown that factors related to the ASCT treatment itself, such as graft source and *in-vivo* T-cell depletion, may have a significant effect on TREC levels, and can subsequently affect outcome. These findings also support the use of TREC measurement as part of the standard repertoire of immunological monitoring after ASCT.