INCREASED CD4+CD25HIGH+ REGULATORY T-CELL ARE ASSOCIATED WITH DISEASE RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) FOR CHRONIC MYELOID LEUKAEMIA (CML)

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The success of SCT as CML largely relies on the graft versus leukaemia (GvL) effect exerted by donor T-cells. CD4+CD25+ regulatory T-cells (Tregs) play a crucial role in the maintenance of peripheral tolerance and have been tested in animal models to successfully prevent GVHD. The role of Tregs in clinical transplantation remains unclear, insofar as the few studies published to date have reported controversial results regarding GvHD. Although there is emerging evidence that Tregs are associated with a poor outcome in cancer patients, none of these studies has investigated the role of Tregs in leukaemia relapse post-SCT.

To address this question we quantified CD4CD25HIGH regulatory T-cells in post-SCT patients and correlated their levels with clinical outcome.

We performed a cross-sectional study at a single institution. We enumerated and characterised peripheral blood CD4+ T-cells and 20 samples from newly diagnosed CML patients. BCR-ABL/ABL ratio was determined in every sample by real-time PCR.

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Patients were considered in remission if the ratio was less than 0.02% and in relapse if higher. All quoted p-values are two-sided with P < 0.05 considered statistically significant.

Patients after SCT had higher levels of Treg than normal donors (median 1.5% vs 0.87, P < 0.01) and untreated CML (median 1.5% vs 0.27, P < 0.0001). In the multiple regression analysis only the time post SCT (before or after 18 months) and disease status (molecular remission versus relapse) were predictive for increased Tregs (Coef = -2.994, P = 0.004 and Coef = -2.395, P = 0.020 respectively). No association with Treg levels and Gemcitabine was found. The logistic regression analysis performed in 43 patients that had not received DLI post SCT confirmed that increased Tregs, both as percentage or absolute numbers, were the only predictive variable for relapse (Exp 1.44, P = 0.011).

A substantial expansion of Tregs occurs early after allogeneic SCT and the presence of high numbers of Tregs 18 months after transplant is predictive of leukaemia relapse. Although the increase might initially have an advantageous effect on graft rejection, our data suggest that Tregs exert an inhibitory effect on GvL.

Preserved Anti-Viral Responses and Improved Survival in Steroid Refractory GVHD Using a Combination of Daclizumab and Infliximab

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Steroid refractory acute graft versus host disease (SRGvHD) is a life threatening complication of SCT with poor prognosis due to infection related mortality. We investigated the combined use of Daclizumab, a humanised monoclonal antibody (Mab) targeting IL-2 and Infliximab, a chimeric human/mouse anti-TNF α, in order to selectively delete alloreactive cells and target 2 different points in the cytokine cascade responsible for aGVHD.

Fifteen consecutive children (median age 4.5 years) with SRGvHD (defined as aGVHD that failed to improve after 1 week of at least 2mg/kg/day of Methylprednisolone) were treated. Donors were MUD or MSD of MUD (n = 9) and mismatched UD (n = 5). All 15 patients had involvement of the skin, 14 of the lower gut and 5 of the liver. All patients had grade 3 (n = 5) or 4 (n = 10) GVHD. Patients were treated with a combination of Daclizumab (1mg/kg, days 1,4,8,15,22) and Infliximab (10mg/kg, days 1,8,15,22), with rapid reduction of steroid dosage to 1mg/kg. Median time of starting the Mabs was 1 day from the onset of GvHD. All children received anti-fungal prophylaxis and prospective viral monitoring.

12/15 patients responded (7 CR, 5 PR), with a median response time of 13 days. Two patients developed recurrent GvHD and received a second course of Mabs. There were 10 episodes of viral reactivations (CMV 4, adenovirus 3, EBV 3) and 3 patients developed probable fungal infections. Impressively, however, there were no infection related deaths. T-cell responses to CMV after mAb infusion were assessed in 5 patients using the IFN-γ ELISPOT assay. As shown in the table below, 4 patients showed a significant response to CMV (defined as > 40 SFC/2 × 10^5 PBMC) in the first 3 months after treatment and at least 1 patient (UPN2) was able to mount a de novo response to CMV after mAb therapy. At a median follow-up of 30 months, 10/15 (66%) children are alive.

35 HISTONE DEACETYLASE INHIBITORS INDUCE INDOLEAMINE 2, 3-DIOXYGENASE AND MODULATE DENDRITIC CELL FUNCTIONS

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Recent studies have demonstrated that suberoylanilide hydroxamic acid (SAHA), a histone deacetylase (HDAC) inhibitor, reduces experimental acute graft-versus-host disease (GVHD). We investigated the cellular-molecular mechanisms of immunomodulation by two HDAC inhibitors, SAHA and ITF 2357. Stimulation of bone marrow derived dendritic cells (DCs) with various TLR ligands (lipopolysaccharide (LPS), PGN, CpG) after pretreatment with either SAHA caused a significant reduction in the secretion of TNF-α, IL-12p70 and IL-6 compared to the untreated controls (P < 0.01). Similar effects were seen using human peripheral blood mononuclear cell derived DCs. Pre-treatment of DCs also significantly reduced their in vitro and in vivo stimulation of allogeneic T cells as measured by proliferation and IFN-γ production (P < 0.05), which was not reversed by anti-IL-10 or anti-TGFβ. No significant difference was observed in the viability of pretreated and control DCs. Pretreatment significantly suppressed the expression of CD40 and CD80. When mixed with normal DCs at 1:1 ratio, SAHA treated DCs suppressed allogeneic T cell responses in a contact dependent manner. When DCs from B6 MHC Class II deficient (H-2b) were treated with SAHA and co-cultured with wild type B6 DCs along with purified allogeneic BALB/c (H-2b) CD4+ T cells in an MLR, the allo-CD4+ T cells proliferated demonstrating the regulation to be dependent on contact between SAHA treated DCs and T cells. To determine the molecular mechanism, we analyzed for the expression of indoleamine 2, 3-dioxygenase (IDO) and found that both SAHA and ITF 2357 increased its expression. To further confirm the relevance of this suppression, we utilized the [BALB/c × B6] model of acute GvHD. B6 animals received 11Gy on day -1 and injected with of 5 million B6 SAHA treated or control DCs
DONOR-REACTIVE THYMIC-DEPENDENT TH1 CELLS CAUSE CHRONIC GVHD

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Chronic graft-versus-host disease (GVHD) continues to be the most common late complication after allogeneic bone marrow transplantation (BMT) and the basic pathophysiology of chronic GVHD remains poorly defined. We have previously demonstrated that donor bone marrow-derived T-cells differentiated via impaired thymus of the recipients cause chronic GVHD after allogeneic transplantation of T-cell-depleted bone marrow (TCD BMT) and the basic pathophysiology of chronic GVHD. In contrast, recipients of CD4+CD25+Foxp3+ regulatory T cells were comparable between WT and [H2-Ab1-/] B6 (H-2b) mice developed GVHD showing clinical and histopathological features of human chronic GVHD. We further studied mechanism of chronic GVHD in this model. A flowcytometric analysis showed that the numbers of CD4+CD25 Foxp3 regulatory T cells were comparable between WT and [H2-Ab1-/] B6 (H-2b) mice. Thymectomy prevented chronic GVHD, thus suggesting the causal association of the thymus. We then investigated reactivity of pathogenic CD4+ T cells in [H2-Ab1-/] C3H mice. A flowcytometric analysis of cell division of CFSE-labeled donor T cells demonstrated that [H2-Ab1-/] C3H CD4+ T cells divided to B6 stimulators, but not to C3H stimulators. A flowcytometric analysis of intracytoplasmic IL-4 and IFN-g suggested polarization of these cells toward Th1 cytokine responses. Adoptive transfer of CD4+CD25 Foxp3 regulatory T cells caused severe and lethal disease in B6 recipients. All mice died by day 40 post-BMT and a pathological analysis of the liver and intestine showed standard pathological features of chronic GVHD. In contrast, the transfer of [H2-Ab1+/] C3H CD4+ T cells did not cause GVHD in C3H recipients. Thus, both in vitro and in vivo experiments demonstrated that these pathogenic CD4+ T cells were primarily B6 reactive. Interestingly, the transfer of these cells caused chronic GVHD in C3H recipients similar to the primary [H2-Ab1+/] C3H mice in the presence of B6-derived antigen-presenting cells (APCs). To further confirm the requirement of B6-derived APCs for the disease, we created WT and C3H chimeras. The transfer of [H2-Ab1+/] C3H CD4+ T cells caused chronic GVHD only in the [B6 → C3H] chimeras, but not in the [C3H → C3H] chimeras, thus confirming the requirement of B6-derived APCs for the transmission of the disease to secondary recipients. These results suggest that self-reactivity of donor Th1 cells play a role in this chronic GVHD.

THE ALLO-REACTIVITY OF DONOR T-CELLS IS SHAPED BY THE PRESENCE OF IMMUNOSTIMULATORY OR IMMUNOSUPPRESSIVE DONOR DENDRITIC CELL SUBSETS IN THE GRAFT: IMPLICATIONS FOR GVHD AND GVLM IN BMT

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The immunological activity of donor T-cells following allogeneic BMT is determined by the initial interaction of T-cells with antigen presenting cells (APC) in the recipient. While previous reports have established a requirement for host APC in the initiation of GVHD, we have explored the role of donor APC as positive and negative regulatory elements in the activation of allo-reactive donor T-cells. We have previously published that allogeneic donor BM enriched for CD11b+ dendritic cells (DC) promotes enhanced donor T-cell chimerism and GVl, suggesting that donor DC subsets modulate immune reconstitution post-BMT (Li, Waller. BBMT 2004). In this study, we examined the immune polarization capabilities and co-stimulator expression of FACS-purified CD11b+ and CD11b- DC subsets in allogeneic BMT and their effect on survival, GVl, GVHD, and immune reconstitution. Fifty thousand purified CD11b+ DC or CD11b- DC from C57BL/6 donors were transplanted along with 5,000 purified Lin- Sca-1, CD8a, CD11b- hematopoietic stem cells (HSC) and 300,000 congenic spleen T-cells into C57BL/6 /H-2Kb and B10BR /H-2Kb recipient mice. The cytokine production, immune proliferation, and Ki-67 expression of CFSE-labeled donor T-cells were evaluated. Additionally, DC subsets were analyzed by flow cytometry for co-stimulator expression, including PD-L1, PD-L2, CD80, and CD86. In tumor free mice, recipients of HSC alone or HSC combined with CD11b+ DC or CD11b DC did not differ in survival for GVHD over 100 days post allo-BMT. Recipients of CD11b DC had increased GVl and donor T-cell proliferation at days 10, 30, 60, and 105, resulting in full-donor T-cell chimerism. In contrast, recipients of CD11b+ DC had mixed T-cell chimerism and increased numbers of immunosuppressive donor CD4+CD25 Foxp3 T-cells. Phenotypic and functional analysis of CD11b+ and CD11b DC revealed that CD11b+ DC had higher levels of immunosuppressive co-stimulators PD-L1, PD-L2, and LBRM, while CD11b- DC induced higher levels of IL-12 and IFN-g when co-cultured with syngeneic T-cells. In conclusion, donor CD11b+ DC promote immune reconstitution and GVl and polarize donor T-cells to Th1 immune responses, while donor CD11b- DC suppress immune reconstitution by expressing suppressive co-stimulators and promoting tolerant donor Treg cells.

DONOR GRAFT CELLS AND SURVIVAL

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Number of Mice</th>
<th>DC Subset</th>
<th>Tumor Cells</th>
<th>Survival (100 days post-BMT)</th>
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<tbody>
<tr>
<td>HSC alone</td>
<td>40</td>
<td>none</td>
<td>none</td>
<td>90%</td>
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<td>20</td>
<td>none</td>
<td>LBRM</td>
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<tr>
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<td>80%</td>
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<td>DC</td>
<td>20</td>
<td>CD11b+</td>
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</tbody>
</table>

HSC = 5,000 Lin- Sca-1+, c-kit+ bone marrow cells; T-cells = 300,000 splenic T-cells; DC = 50,000 FACS sorted CD11b+ or CD11b+ dendritic cell subsets; LBRM = 100,000 T-lymphoblastic leukemic cells; *** p<0.01 compared to other groups with LBRM.

PREDICTION OF ACUTE GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION BY A PROTEOMIC AGVHD-SPECIFIC PATTERN

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Oral Presentations