ELEVATED LEVELS OF CD19+ CD21- TRANSITIONAL B CELLS IN CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) ASSOCIATED WITH ELEVATED PLASMA BAFF LEVELS AND BAFF RECEPTOR EXPRESSION: Rehman, N.K., Dickinson, J., Bashar, S., Rader, C., Pacilet, S.Z., Gress, R.E., Hakim, F.T. National Cancer Institute, NIH, Bethesda, MD.

B Cell Activating Factor (BAFF) also termed BlyS) is a critical factor in the survival, differentiation and function of B cells, but elevated plasma levels of BAFF have been associated with the development of B-cell mediated autoimmunity, both in murine models and in humans. BAFF administration–induced autoimmunity has been linked to increased survival of the transitional B cell population, resulting in a failure to eliminate auto-reactive B cell populations by negative selection during B cell maturation. Murine studies had determined that mice BAFF deficient (BAFF-/-) mice had both an unmobilized donor lymphocyte population of 15 individuals (Mann Whitney p < 0.0001). By multi-parameter flow cytometry we further determined that the median percentage of CD19+ CD21- transitional B cells in 71 CGVHD patients was significantly higher than that in 40 normal adult donors (Mann Whitney p < 0.0001). Following transplantation, the B cell population is reconstituted primarily by maturation of new B cells from the marrow, hence an elevated frequency of transitional B cells was observed. The CD19+ CD21- transitional B cells was significantly higher in those patients with higher BAFF levels (p = .001). Elevated frequencies of CD21- B cells in CGVHD patients may therefore reflect elevated BAFF levels. Altered transitional B cell survival may contribute to the pathogenesis of auto-reactivity by the survival of auto-antigen reactive immature B cells.

Cell Population Ki-67 Proliferation Index

<table>
<thead>
<tr>
<th>Treg (CD45RA- CD27-),%</th>
<th>CD4+ T cell</th>
<th>CD8+ T cell</th>
<th>Non-T cell Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8.2 ± 2.5</td>
<td>2.0 ± 0.7</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>GCSF</td>
<td>6.2 ± 2.6</td>
<td>1.5 ± 0.7</td>
<td>2.9 ± 2.5</td>
</tr>
<tr>
<td>t-test</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Mean ± 1 SD (95% CI).

PHASE-II STUDY OF INFliximAb FOR THE PROPHYLAXIS OF ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) FOLLOWING ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSC): Hamadani, M.1, Phillips, G.N.2, Elder, P.M.1, Jansak, B.1, Rhein, W.J.1, Penza, S.1, Lin, T.S.1, Farag, S.S.1, Desme, S.M.1,1, Arthur G. James Cancer Hospital, Ohio State University, Columbus, OH; 2Arthur G. James Cancer Hospital, Ohio State University, Columbus, OH; 3Indiana University School of Medicine, Indianapolis, IN.

Introduction: Infliximab is a chimeric monoclonal antibody that binds with high affinity to the soluble and transmembrane forms of tumor necrosis factor-α, and inhibits their binding with the cellular receptors. A number of retrospective studies have shown activity of this drug in the treatment of steroid refractory acute (a)GVHD. We conducted a prospective phase-II trial of infliximab for prophylaxis of aGVHD following AHSC. Methods: Selection criteria included age >20 yrs, sibling (Sib) or unrelated donor (URD) availability and myeloablative (MA) AHSC for hematologic malignancies (except CML in 1st chronic phase and aplastic anemia). Prophylaxis for aGVHD consisted of infliximab (10 mg/kg/dose) given 1 day prior to starting MA conditioning and subsequently on days 0, +7, +14, +28 and +42, cyclosporine and methotrexate (15 mg/m² day 1 & 10 mg/m² days 3,6,11). Results: Nineteen patients (pts) were prospectively enrolled. There were 13 male and 6 female pts with a median age of 57 yrs (range 27–64 yrs). Diagnoses included AML/MDS (n = 11), NEL (n = 4) and ALL (n = 4). Donors included matched Sib (n = 14), matched URD (n = 4) and mismatched URD (n = 1). 15 pts received MA conditioning with busulphan/cyclophosphamide, while 4 received TBI. All pts received peripheral blood stem cells. Prospectively enrolled pts getting infliximab (IG) were compared with a matched control group (CG) (n = 30). Pts in IG were matched for age, di- gender, donor type, HLA typing, GVHD prophylaxis, conditioning regimen and stem cell source with IG. Median number of CD34+ cells in IG and CG was 4.95 and 5.22 × 106 cells/kg of
recipient respectively. Cumulative incidence of grade II-IV aGVHD in IG and CG was 25% and 35% respectively (p > 0.05). No difference was seen in days to onset of aGVHD between the two groups. Patient age, sex, donor type and conditioning regimen had no effect on the probability of response to infliximab. Rate of chronic GVHD, the pathology and CGre of cGVHD were 84% and 61% respectively (p = 0.22). Ps in IG had significantly more fungal infections (n = 5) compared to CG (n = 1) (p = 0.02). Kaplan-Meier estimates of 3 yr overall survival for IG and CG were 31% and 38% respectively (p = 0.42). Estimates of 3 yr progression free survival are 59% and 56% in similar order (p = 0.68). Conclusion: In conclusion, infliximab does not reduce incidence of grade II-IV aGVHD compared to historic controls. It causes a significant increase in fungal infections and may increase the likelihood of chronic GVHD.

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Graft-versus-host disease: a minor-mismatched mouse model with gradual progression from the acute to the chronic phase
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Chronic graft-versus-host disease (cGVHD) is the most frequent long-term complication after allogeneic hematopoietic cell transplantation (HCT). While acute GVHD (aGVHD) is largely donor T cell (TC) induced, the pathophysiology of cGVHD remains unclear. Amongst the issues to be clarified are the identification of the TC subsets that drive the pathophysiology, the delineation of antigen targets and the role of B cells (BC). Most experimental models study acute GVHD, whereas models for the chronic phase are scarce. C57BL/6 (H2b)BALB. B (H2db) is an established minor-mismatched mouse model for aGVHD. Here, we assessed whether mice also develop chronic long-term symptoms. Lethally irradiated recipients were given FACs purified hematopoietic stem cells (HSC: cKit+/Thy1.1Lin-Sca1+). For induction of GVHD titrated doses of splenocytes (SP; 5 × 10^5–1 × 10^6) or pul- rified CD4+ or CD8+TC were co-transferred. Mice were followed over an extended time (1 y) for clinical signs, weight loss, histology, chimerism, and IgG synthesis (donor/host). Recipients of pure HSC remained healthy, steadily increasing in weight (107% of baseline (BL); 100). Mice given SP developed aGVHD with mor- bidity and mortality correlating to the SP dose. Survivors of the acute phase stabilized by d50–70, with only subtle signs for months, and weight remained below BL (d100: 95%/85% for 10^6/10^7 SP, re- spectively). Ultimately, they developed a full-blown picture of cGVHD, with erythroosquamous skin lesions, alopecia, cirrhotic liver changes, and conjunctivitis at 1 y post-HCT. The most re- markable histological changes were inflammatory portal liver infiltrates, which gradually progressed to fibrosis and complete disrup- tion of a regular cell pattern. Intestines were primarily affected in the acute phase, whereas skin changes (subcutaneous atrophy, in- filtration of hair follicles) manifested later. CD4, but not CD8+TC induced the full picture of aGVHD. Recipients of HSC remained mixed chimeras, addition of SP promptly converted recipients to full donor chimeras. Delays in BC reconstitution correlated with the degree of aGVHD. Despite this, high levels of donor IgG syn- thesis were observed. In conclusion, R6 → BALB. B is a valuable model to study GVHD with convincing histological signs evolving from acute changes. Mouse studies delineating the GVHD-induced TC subsets will improve our understanding of the pathophysiology of GVHD, and will be an essential piece paving the way of graft engineering.

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The PTPN22 1858C/T polymorphism is associated with the development of grade 3 to 4 acute graft-versus-host disease after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning
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Graft-versus-host disease (GVHD) is a cause of considerable morbidity and mortality after allogeneic hematopoietic cell transplan- tation (HCT) following nonmyeloablative conditioning. Ge- netic polymorphisms in various genes, associated with the immune system have been implicated in the development of GVHD. The PTPN22 gene encodes LYP, which is involved in sup- pression of T-cell receptor signalling. The 1858 C→T polymor- phism in PTPN22, renders T-cells hyperresponsive, and the 1858 T allele has been implicated in conferring increased susceptibility to various autoimmune diseases. As in autoimmunity, T-cell activation plays a key role in the development of GVHD. To assess the in- fluence of the PTPN22 1858 C→T polymorphism on development of GVHD after allogeneic HCT following nonmyeloablative condi- tioning, 100 consecutive patient-donor pairs receiving allogeneic HCT with related (n = 66) or unrelated (n = 34) donors for hema- tological malignancies (HD: 51, MM: 12, NHL: 17, MDS: 18, AML: 24, CML: 5), between March 2000 and December 2005 at Rigshospitalet, Denmark, were genotyped. The cumulative incidence of grade 2–4 acute GVHD, grade 3–4 acute GVHD and extensive chronic GVHD was 67%, 24%, and 49%, with no difference between patients carrying the C/C or C/ T and T/T genotype or donors carrying the C/C or C/T and T/ T genotype. To assess a possible gene-dosage effect, the number of T-alleles in each recipient-donor pair was cumulated, and the cu- mumulative incidence of grade 3–4 acute GVHD increased from 20% in recipient-donor pairs carrying no or one T-allele to 50% in recipient-donor pairs carrying two or more T-alleles (p = 0.04), while there was no difference in grade 2–4 acute and extensive chronic GVHD between groups. In the competing risk regression analysis, the recipient-donor pair genotype with 2 or more T-alleles was an independent risk factor (hazard ratio 3.0; 95% CI 1.2–7.5) for de- velopment of grade 3–4 acute GVHD, even after adjusting for baseline variables known to affect GVHD rates. Furthermore, patients from recipient-donor pairs carrying two or more T-alleles were hospital- ized for more days (p = 0.01) due to GVHD (median = 15 days; range 0–63 d), than patients from recipient-donor pairs with no or one T-allele (median = 0 days; range 0–104 d). Collectively, our data suggest, that the PTPN22 1858 C→T polymorphism, when present in both recipient and donor, is a risk factor for development of grade 3–4 acute GVHD after nonmyeloablative condi- tioning allogeneic HCT.

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Immune activation with interleukin-2 and granulocyte- macrophage colony stimulating factor for treatment of relapse after allogeneic stem cell transplantation

Donor lymphocyte infusion (DLI) is used for relapse after allogeneic stem cell transplant (ASCT). Immune activation with cytokines maybe an alternative to DLI. We studied the use of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Interleu- kin-2 (IL-2) for immune activation at the time of relapse after ASCT in patients (pts) with hematologic malignancies. Pts. received subcutaneous GM-CSF at 500 mcg/day on days 1–14 and IL-2 at 1 × 10^6 units/m2/day on days 8–14. Pts. were off immunosuppressive therapy and had no prior history of graft versus host disease (GVHD) at the start of treatment. Twelve pts. received IL-2/ GM-CSF for treatment of relapse AML (7), ALL (2), CML (1), MDS (2). Median age was 55 (range 8–66). Stem cell sources included: peripheral blood = 9, bone marrow = 2, umbilical cord blood (UCB) = 1. Donor sources were: match-related sibling = 4 and match-unrelated donor = 8 (UCB = 1). Nine pts. had resistant relapse or primary resistant disease at time of ASCT. Median time from transplant to relapse was 4 months (range = 1–14). Two pts. had failed DLI and 5 pts. had received reinduction chemotherapy prior to IL-2/GM-CSF. Eight pts. responded to IL-2/GM-CSF (CR/7, PR = 1). Two pts. relapsed. Disease free survival after aGVHD was the same as post IL-2/GM-CSF. Six pts. developed GVHD and of these 4 were responders. Two pts. had GM-CSF discontinued due to in- crease in peripheral blood blasts. No other toxicities related to