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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

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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 human disease. In mice 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 only CD21+ T2/T3 transitional B cells expressed the BAFF receptor and could be rescued by elevated BAFF levels. In contrast, in man, we have determined that the more immature circulating CD19++CD21– T1 transitional B cell subpopulation expresses the BAFF-R to the same degree as mature B cells in both normal donors and patient populations. We therefore concurrently investigated both plasma BAFF levels and CD19++CD21– T1 transitional B cell frequencies in patients entered into an ongoing NCI CGVHD natural history protocol. We determined that plasma BAFF levels were elevated in many CGVHD patients as compared with those in normal donors (Mann Whitney $p < 0.0001$). By multi-parameter flow cytometry we further determined that the median percentage of CD19++CD21– T1 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 would be expected when mature B cell levels are low; transitional CD19++CD21- B cell frequencies remain elevated over normal, however, even in CGVHD patients with B cell levels greater than 100 cells/microliter ($p = .003$). Comparison of patients in the upper and lower 50th percentile of plasma BAFF levels determined that the frequency of 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 later generation of pathologic auto-reactivity by the survival of auto-antigen reactive immature B cells.

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GCSF DECREASES CD4+CD25+CD127LO REGULATORY T CELL PROLIFERATION INDEX IN STEM CELL DONORS

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Increasing the number of regulatory T cells (Tregs) in donor grafts prevents acute graft-versus-host disease (aGVHD) in murine models of hematopoetic stem cell transplant (HSCT). It has been reported that filgrastim (G-CSF) mobilization increases the proportion of donor graft CD4+CD25+ T cells. This finding is bolstered by evidence from murine models, and by in vitro systems demonstrating an impaired response to mitogenic stimulation after in vivo G-CSF. We can now more precisely identify human Tregs by the markers CD127lo and Foxp3. To test the hypothesis that G-CSF increases the proportion of Tregs in human HSC donors, we phenotyped the donor graft T cell population of 15 individuals who had both an unmobilized DLI collected by apheresis and a separate G-CSF mobilized graft. Our phenotyping included CD4, CD25, CD127, Foxp3, CD8, markers of memory differentiation (CD45RA, CD27) and the cell cycle protein Ki-67. G-CSF did not alter the total proportion of CD4+CD25+CD127lo Tregs in the CD4 compartment, % Tregs baseline (mean = 4.82, SD = 1.08, 95% CI = 4.30–5.42), % Tregs after G-CSF (mean = 5.08, SD = 1.22, 95% CI = 4.40–5.75). When we examined the cycling fraction of various lymphoid subsets, we first observed that at baseline, Tregs had a much higher proliferative index (%Ki-67+) than other blood subpopulations. Furthermore, proliferation within the Treg compartment decreased significantly following G-CSF treatment (Table 1). We also found a G-CSF treatment-related decrease in proliferative index in the (non-Treg) CD4+ compartment and a trend toward increased proliferation within the B/NK compartment. To exclude direct effects of G-CSF on T cells we tested for the presence of CD114 (G-CSFR) on T cells by flow cytometry, and found none. Furthermore G-CSF treatment of PBMC (50 ng/mL for 5 d) did not influence the proliferative index of conventional CD4+, CD8+, or Tregs after in vitro T cell stimulation, suggesting that the mechanism by which G-CSF reduces Treg proliferative index in HSC donors is indirect. Since depletion of the host lymphoid population via conditioning presumably facilitates the expansion of transferred donor graft lymphocytes, it will be important to test if variations in the proliferative index of transferrred Tregs or other lymphoid subsets results in an altered "proliferative momentum" in HSCT recipients, which might influence the rate of Treg recovery and, potentially, aGVHD rates after HSCT.

Cell Population Ki-67 Proliferation Index

	Treg (CD4+ CD25+ CD127 lo)	CD4+ T cell	CD8+ T cell	Non-T cell Lymphocytes (B cell & NK)
Baseline	8.2 ± 2.5 (6.8, 9.6)	2.0 ± 0.7 (1.6, 2.5)	2.3 ± 1.0 (1.7, 2.8)	5.1 ± 1.6 (4.2, 6.0)
GCSF	6.2 ± 2.6 (4.7, 7.7)	1.5 ± 0.7 (1.1, 1.9)	2.9 ± 2.5 (1.5, 4.3)	11.2 ± 12.1 (4.5, 17.9)
t-test (paired)	<0.0001	0.01	0.36	.057

Mean ± 1 SD (95% CI).

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PHASE-II STUDY OF INFLIXIMAB FOR THE PROPHYLAXIS OF ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)

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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 AHSCT. **Methods:** Selection criteria included age >20 yrs, sibling (Sib) or unrelated donor (URD) availability and myeloablative (MA) AHSCT 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/m^2 day 1 & 10 mg/m^2 days 3,6,11). **Results:** Nineteen patients (pts) were prospectively enrolled. There were 13 male and 6 female pts with a median age of 53 yrs (range 27–64 yrs). Diagnoses included AML/MDS (n = 11), NHL (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 CG were matched for age, diagnosis, 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. Rates of chronic GVHD in IG and CG were 84% and 61% respectively ($p = 0.22$). Pts 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 INTO 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 (B6)→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+Thy1.1loLin-Sca1+). For induction of GVHD titrated doses of splenocytes (SP; 5×10^5–1×10^7) or purified 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); d100). Mice given SP developed aGVHD with morbidity 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: 93%/83% for $10^6/10^7$ SP, respectively). Ultimately, they developed a full-blown picture of cGVHD, with erythrosquamous skin lesions, alopecia, cirrhotic liver changes, and conjunctivitis at 1 y post-HCT. The most remarkable histological changes were inflammatory periportal liver infiltrates, which gradually progressed to fibrosis and complete disruption of a regular cell pattern. Intestines were primarily affected in the acute phase, whereas skin changes (subcutaneous atrophy, infiltration of hair follicles) manifested late. 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 synthesis were observed. In conclusion, B6 → BALB. B is a valuable model to study cGVHD with convincing histological signs evolving from acute changes. Mouse studies delineating the GVHD-inducing 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 transplantation (HCT) following nonmyeloablative conditioning. Genetic 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 suppression of T-cell receptor signalling. The 1858 C→T polymorphism 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 asses the influence of the *PTPN22* 1858 C→T polymorphism on development of GVHD after allogeneic HCT following nonmyeloablative conditioning, 100 consecutive patient-donor pairs receiving allogeneic HCT with related ($n = 66$) or unrelated ($n = 34$) donors for hematological malignancies (HD = 13, MM = 14, CLL = 12, NHL = 17, MDS = 18, AML = 24, CML = 2), 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 cumulative 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 development 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 hospitalized 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 conditioning 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

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Donor lymphocyte infusion (DLI) is use 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 Interleukin-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/m^2/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. remain disease free at 18 and 26 months post IL-2/GM-CSF. Six pts. developed GVHD and of these 4 were responders. Two pts. had GM-CSF discontinued due to increase in peripheral blood blasts. No other toxicities related to