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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