produce large amounts of interferon-gamma (IFN-g) under inflammatory conditions. Here we address the role of IFN-g production by Tregs in the course of experimental GvHD.

Methods: In a C57BL/6 into BALB/c mouse model of acute GvHD we monitored the intracellular cytokine expression of Tregs using FoxP3-reporter mice (C56BL/6) and congenic markers. We addressed the role of IFN-g in experimental GvHD i) by employing the IFN-g blocking mAb XMG1.2 and ii) by adoptive transfer of Tregs and/ or effector T cells purified from ifng–/– mice. GvHD severity was monitored by survival, clinical score and histological analysis.

Results: Co-transferred Tregs in a C57BL/6 into BALB/c model readily secreted IFN-g but stably remained FoxP3+ and prevented lethal GvHD. Intracellular staining revealed that at day 4 after transplantation approximately 35% of these allogeneic Tregs produced IFN-g. In this experimental setting blocking of IFN-g with mAb completely abolished the protective effect of Tregs and led to early death from exacerbated GvHD. Of note, we also observed a similar fatal outcome of experimental GvHD when we co-transferred ifng–/– Tregs and wild type effector T cells.

Conclusions: Our data suggest that IFN-g should not be regarded as an adverse pro-inflammatory cytokine under the highly inflammatory environment of acute GvHD since it is required for the protective action of Tregs. We hypothesize that this may be due to a deviation of effector T cell profiles towards Th17. In ongoing experiments we therefore address this issue by employing effector T cells from il17a–/– / il17f–/– double knock-out mice.

522

DONOR-LYMPHOCYTE INFUSION IN PATIENTS WITH PERSISTENT OR RECURRENT MULTIPLE MYELOMA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective: The graft-versus-leukemia (GVL) effect of donor-lymphocyte infusions (DLI) is well documented in hematologic malignancies, including multiple myeloma (MM). We evaluated the role of DLI in persistent or recurrent MM after allogeneic hematopoietic stem cell transplantation (allo HCT).

Materials and Methods: We identified 23 patients with MM, who received DLI at UT-MD Anderson Cancer Center between 7/1996 -6/2008. Patients had persistent or recurrent disease after allo HCT, and were treated with DLI collected from their original allo HCT donors.

Results: Nineteen patients received DLI from matched related donors (MRD) and 4 from matched unrelated donors (MUD). Median age at DLI was 51 years (range: 38-62). A total of 33 DLI doses were administered. Seventeen patients received 1 infusion, 4 patients received 2, and 2 patient received 4 infusions. Median interval between allo HCT and the first DLI was 8.2 months (range: 2.9 to 119.5). Median follow up from the first DLI was 18 months (range 1-126). Twenty-six DLI (78%) were given without preceding cytoreductive chemotherapy. The median DLI dose was 3.5 x 10^7 CD3+ T cells (range 0.5 to 14.8 x 10^7). Overall response rate was 30%, with 10 of 33 DLI doses associated with objective clinical responses (3 CR, 5 VGPR, 2 PR). Thirteen (39%) additional DLI were followed by stable disease. Median response duration was 5 months (range 2-10). ORR to DLI in relapsed disease was 10% (2/20), compared to 61% (8/13) for residual disease ($p = 0.0048$). Grade II-IV acute graft-versus-host disease (GVHD) was seen in 6 (26%) patients, with median onset of 5 weeks from DLI. None of the patients developed chronic GVHD after DLI. Median overall survival from the first DLI was 18.6 months.

Conclusions: DLI is associated with objective clinical responses patients with relapsed or persistent MM, with a significantly higher response rate in patients with persistent vs. relapsed disease. The risk of acute GVHD was low. The role of DLI needs to be further explored in prospective clinical trials for patients with relapsed or persistent MM.

523

KINETICS OF MURINE GVHD INDUCTION ACROSS MINOR AND MAJOR HISTOCOMPATIBILITY BARRIERS

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It is known that Graft-vs-Host Disease (GVHD) occurs in response to minor histocompatibility antigens, but little is known about the kinetics of donor T cell proliferation and homing in minor mismatch models of allogeneic hematopoietic cell transplantation (HCT). This is in contrast to models across major histocompatibility barriers, where early development of GVHD has been better characterized. Yet, because minor mismatch models are more similar to clinical HCT, it is critical to understand how GVHD develops across minor barriers. To investigate temporal and spatial events of donor T cell activation and homing, side-by-side transplants were conducted using bone marrow and enriched CD4 and CD8 T cells (Tcon) from donor C56BL/6 mice ($H2^b$) into either major mismatched Balb/c ($H2^d$), or minor mismatched Balb.b ($H2^b$), irradiated recipients. Balb/c mice received 1x10^6 Tcon while Balb.b mice received 15x10^6 Tcon, based on titration experiments. Proliferation and migration of donor Tcon was monitored using *in vivo* and *ex vivo* bioluminescent imaging, and CFSE labeling. Expression of T cell activation and homing markers was examined using flow cytometry analysis of donor CD4 and CD8 cells re-isolated from transplanted mice. Donor Tcon from Balb.b mice exhibited significantly reduced proliferation at both 3 and 6 days post transplant ($p < 0.01$, $n = 43$). But, mirroring our earlier findings in major mismatch models, donor Tcon in the minor model homed to nodal sites by day 3, followed by an exit to tissues by day 6, albeit reduced. No significant differences in the expression of the activation and homing markers examined were noted by day 3, although there was variation across tissues and between CD4 and CD8 cells. By day 6, donor T cells re-isolated from Balb.b recipients had reduced levels of $\alpha_4\beta_7$ and P-selectin, and increased retention of CD62L. These data support the idea that early events of donor T cell activation, particularly spatially, are similar across minor and major histocompatiblity barriers, reinforcing the usefulness of both models as translational research tools. More importantly, these data suggest that delays in visible GVHD onset in minor mismatch tranpslants arise from temporal differences in the effector phase of T cell action, rather than delays in the initiation phase. These findings support targeting very early events in T cell activation as the most effective method of reducing GVHD in clinical settings.

524

ROLE OF CLINICAL LABORATORY MARKERS OF INFLAMMATION IN ASSESSING CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) ACTIVITY AND SEVERITY

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Background: cGVHD is a major cause of health problems and mortality after allogeneic HCT. Routine clinical laboratory parameters are established in outcomes monitoring of systemic inflammatory or autoimmune disease, but their value in cGVHD is unknown. To determine the relationship between laboratory markers of

Table 1. Correlation between laboratory parameters and categorical outcomes

Parameter (units) median, [range]	CRP (mg/L)	C3 (mg/dL)	(mg/dL)	IgG (mg/dL)	HGB (g/dL)	Lymph (K/uL)	Platelets (K/uL)
Intensity of immunosuppression							
none/mild (n=49)	0.73 [0.3-44]	129 [66-179]	24 [15-37]	887 [200-3380]	13.3 [10.7-17.1]	1.63 [0.34-7.55]	n.s.*
moderate (n=71)	0.69 [0.15-41.6]	128 [64-210]	27 [13-61]	570 [98-2190]	12.5 [8.2-16.1]	1.22 [0.11-5.00]	n.s.
high (n=69)	1.22 [0.3-58.9]	147 [76-216]	31 [13-74]	580 [142-2050]	12.3 [8.9-16.2]	1.00 [0.15-5.30]	n.s.
p-value	0.008	0.0046	0.0011	0.002	0.0006	0.011	
Activity by therapeutic intent							
non active (n=84)	0.61 [0.15-44.3]	126 [64-210]	24 [13-74]	n.s.	n.s.	n.s.	223 [33-465]
active (n=71)	1.73 [0.3-58.9]	145 [76-216]	31 [15-68]	n.s.	n.s.	n.s.	278 [56-648]
p-value	<0.0001	0.0003	0.0004				0.012
NIH global severity stage							
moderate (n=62)	0.62 [0.15-27]	122 [66-187]	n.s.	n.s.	n.s.	n.s.	214 [33-461]
severe (n=125)	1.15 [0.26-58.9]	139 [64-216]	n.s.	n.s.	n.s.	n.s.	265 [34-648]
p-value	<0.0001	0.0017	n.s.	n.s.	n.s.	n.s.	0.0028

*Not significant.

inflammation and cGvHD severity and activity we analyzed a large prospective patient cohort.

Methods: 189 adults were enrolled onto the NCI cross-sectional cGvHD natural history study between 2004-2010. 33% had moderate and 66% had severe cGvHD per NIH criteria (88% classic, 12% overlap). 80% were receiving systemic immunosuppression and failed a median of 4 (range 0-9) prior therapies (PST). Median follow-up of survivors was 30.3 months (1-70). Laboratory predictors included: CRP, ferritin, complement, albumin, IgG, β-2 microglobulin, total protein, PTH (parathyroid hormone), ESR, CBC and platelets. cGvHD severity outcomes included: NIH global stage (moderate vs. severe), NIH average organ score, Lee symptom scale, SF36 physical scale, Schirmer's tear test, Schubert oral mucositis scale (OMRS), skin body surface area (BSA) as continuous outcomes. cGvHD activity outcomes (categorical) included: intensity of immunosuppression (none/mild, moderate, high), clinician's global assessment (CGA) of change (7-point scale) and active vs. non active based on therapeutic intent at enrollment.

Results: Correlation between continuous outcomes and laboratory parameters was weak. Significant univariate analysis results related to categorical outcomes are shown in the Table. By multivariable logistic regression analysis taking into consideration clinical, demographic and laboratory parameters, lower albumin ($p < 0.0001$), higher platelets ($p = 0.045$) and higher number of PST ($p < 0.0001$) were associated with active disease (identifying 75% of active and 77% of non-active cases). Similarly, higher platelets ($p = 0.021$), higher number of PST ($p < 0.0001$) and lower FEV1 ($p < 0.0001$) were associated with severe disease (identifying 76% of severe and 74% of moderate cases). Only higher absolute lymphocytes ($p = 0.019$), higher IgG ($p = 0.0054$) and lower PTH ($p = 0.045$) were associated with better survival in univariate analysis.

Conclusion: Some common laboratory indicators of inflammation can serve as markers of active and severe cGvHD. Better understanding of biologic mechanisms influencing these markers may lead to deciphering the biology of cGVHD and designing therapeutic approaches.

525

EXPRESSION OF THE CHEMOKINE RECEPTOR CCR5 ON BLOOD CD11C+ CD16+ DENDRITIC CELLS POST-ALLOGENEIC HEMOPOIETIC CELL TRANSPLANT IS PREDICTIVE FOR THE DEVELOPMENT OF ACUTE GRAFT VERSUS HOST DISEASE

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Introduction: Dendritic cells (DC) are centrally involved in the development of acute graft-versus-host disease (GvHD) following allogeneic hematopoietic cell transplantation (alloHCT). We previously showed that the activation status, as assessed by CMRF-44 antigen expression, of blood CD11c+ myeloid DC is highly associated with the severity of acute GvHD, and that activated DC may be detected in the circulation prior to clinical presentation of GvHD (Transplantation 2007;83: 839–846). We also reported that there was also a positive correlation between aGVHD and the expression of the chemokine receptor CCR5 on myeloid DC (Blood, 114,Suppl.:2251,2009). Because of the phenotypic and functional heterogeneity of the CD11c+ DC population, we further investigated the precise nature of the CD11c+ DC subset expressing CCR5 in the peripheral blood in 24 patients post alloHCT, and correlated the findings with GVHD outcomes.

Methods: Peripheral blood was collected twice weekly up to day 100 post transplant from 24 alloHCT patients. The expression of CCR5 receptor on CD11c+ and CD11c- DC subsets was evaluated using multiparameter flow cytometry.

Results: Eleven of 24 patients developed acute GvHD (4 grade I, 7 grades II-IV), the remaining 13 patients had no GvHD. The percentage of CD11c++ CD16+ DC expressing CCR5 correlated with the development of acute GvHD grades II-IV. The maximum CCR5 expression detected on CD11c+ CD16+ DC in patients developing grade II-IV GvHD (mean 36.0 +/− 6.9%, n = 7)was higher than in those with grade 0-I GvHD (17.2 +/− 3.2 %, n = 17) ($p = 0.0153$), and occurred prior to the clinical onset of GVHD in 6 of 8 patients with CCR5 levels > 20%. Levels of expression of CCR5 on other DC subsets, including CD16- CD11C+ DC, were not preditive for GVHD.

Conclusion: Expression of CCR5 on circulating CD11c+ CD16+ myeloid DC post allo-HCT correlates with the development of moderate to severe GvHD. This observation may indicate altered homing patterns of these cells during the alloimuune response. Detection of raised numbers of CCR5+ CD11c+ CD16+ DC could allow earlier therapeutic intervention prior to the development of clinical GVHD, if these findings are confirmed in a larger study.

526

IMPAIRED THYMOPOIESIS WITH NORMAL T REGULATORY CELL NUMBERS IS ASSOCIATED WITH SEVERE CHRONIC GRAFT-VERSUS-HOST DISEASE

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Chronic GVHD (cGVHD) is a significant complication of allogeneic hematopoietic stem cell transplantation (AHSCT). Although T cells have been implicated in cGVHD pathobiology, the role of the thymus in this process has yet to be defined. We characterized thymus and spleen T cell subsets in a murine model of cGVHD to elucidate the role of the thymus in this process. The murine model of minor-histocompatibility mismatch cGVHD, B10.D2- > BALB/c, was validated by pathologic review that correlated with the clinical scoring and showed cGVHD pathology in skin, stomach,