ECP is largely used in Acute and Chronic GVHD patients. Moreover, some clinical studies are ongoing to evaluate further ECP employment in GVHD prevention. Therefore, laboratory and clinical parameters predictive of ECP response would be extremely useful. For instance, recent published data showed great interest on VBTCR oligoclonal CD4 or CD8 subgroups, and T reg CD4+CD25+ CTLA4+/-, which could be used as predictive factors for GVHD outcome.

We present data from seriate, cytfluorimetry phenotype studies performed on 200 blood samples from 4 aGVHD and 13 cGVHD patients that underwent ECP in our Apheresis Unit. 15/17 had skin involvement or oral mucose and/or ocular disease, 3/17 showed liver GVHD, 2/17 had lung function compromised. Schedule for ECP therapy established 6 and 12 months of treatment for Acute or Chronic GVHD, respectively. Clinical evaluation was made every three months and according to known protocols. Skin and oral mucosa improvement was achieved in the 64% of the patients, liver recovery was stabilized in the 30%, while no response was reported in lung function.

Finally, after more than 6 months of treatment, 3/14 cGVHD patients failed to respond and 3/14 maintained stable disease without steroids administration.

A pathological increment of the VBTCR CD4+ or CD8+ oligoclonal (being fixed as > 15% of the circulating lymphocytes) was found in 10/17 patients. VB20, VB16, VB14 and VB3 were the most recurrent oligoclones, but we could find only weak correlations with ECP response-rate, kind and extension of GVHD. At the start of ECP, Treg CD4+CD25+CTLA4+ (mean 0.4% of the circulating CD4+) were detected in all 2 aGVHD and in 1 cGVHD early responders to ECP. This subset was lacking in 3 ECP refractory patients in which, on the contrary, was detectable the subset CD4+CD25+CTLA4+ (mean 1.6% of the circulating CD4+) also detectable in the other 11 remaining patients. Interestingly, in this last group of subjects Treg cells seemed to be correlated to the ECP response rate. In particular, responders showed inverse trends in the CTLA4+ (increasing) or CTLA4- (decreasing) subsets during ECP treatment.

In conclusion, Treg subpopulations seem to be useful as predictive factors of GVHD outcome, in accordance with other studies results. Nevertheless, further research is still necessary in order to draw definitive conclusions.

**330 CARCINOEMBRYONIC ANTIGEN RELATED CELL ADHESION MOLECULE 1 IS AN IMPORTANT SUPPRESSOR OF INTESTINAL AND SYSTEMIC ACUTE GRAFT-VERSUS-HOST-DISEASE**

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Carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM-1) is a transmembrane glycoprotein found on leukocytes, endothelium, and epithelium, and its activation can attenuate colitis in murine models. Microarray analysis revealed that CEACAM-1 expression is increased during gut graft-versus-host-disease (GVHD). We studied the role of CEACAM-1 in a mouse allogeneic bone marrow transplantation model (B6→BALB/c). Technical replicates of 8 plasma measured at 1:50, 1:150, 1:500 provided correlation coefficients, R²=0.94-0.97 confirming technical feasibility. In order to identify targets of allo-Ab responses that develop post-transplant but are absent pre-transplant. ProtoArray™ (Invitrogen) displays 5,000 full-length human proteins with N-terminal GST epitopes expressed in baculovirus and affinity purified under native conditions maintaining their cellular enzymatic activities/native conformations. Technical replicates of 8 plasma measured at 1:50, 1:150, 1:500 provided correlation coefficients, R²=0.94-0.97 confirming technical feasibility. In order to identify targets of allo-Ab, pretransplant fluorescent signal intensities were subtracted from their one-year plasma results for all 5000 antigens. While Ab responses were unchanged for 4600 (92%) antigens, new allo-Ab responses targeted 60-75 antigens with fluorescent differences ranging 0.5 to 3 logs. In comparison with their respective donor results, 30-40% appear to result from adoptive donor transfer while the remaining develop de novo. Over 90% of allo-Ab targets have known non-synonymous SNP which when disparate in donor and recipient may elicit alloimmunity and this genotyping is ongoing. No single protein was recognized by “new” Ab responses in all patients, however polymorphic proteins Growth Arrest Specific-7 (GAS7), laminin A/C, and ribosomal protein S19 (RPS19) were recognized by two of the four patients after HCT. Results from plasma samples collected 3, 6, 9 and 12 months after HCT demonstrate the progression of alloimmune immune responses. Though these novel mHA require validation by large clinically characterized patient samples, protein microarrays are innovative, powerful tools for high-throughput global assessment of B-cell alloimmunity after HCT providing sufficient reproducibility for candidate mHA discovery.

**332 EFFECTIVE GRAFT-VERSUS-LEUKEMIA RESPONSES ARE ASSOCIATED WITH THE PRESENCE OF NUCLEIC ACID-IMMUNOglobulin COMPLEXES THAT STIMULATE TOLL-LIKE RECEPTORS (TLR) 8 AND 9**

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In patients with chronic myeloid leukemia (CML) who demonstrated effective graft-versus-leukemia (GvL) responses after DLI revealed more profound activation of T cells in the CEACAM-1 deficient setting as demonstrated by increased early CD25 expression and CD62L downregulation on fast-cycling alloreactive T cells in the spleen and liver. We found no significant differences in serum levels of TNF-α or interferon-γ, T cell proliferation kinetics upon adoptive transfer, percentages of alloactivated CD4 and CD8 cells, or percentages of CD4+Foxp3+ regulatory T cells in WT recipients of CEACAM-1-/- T cells, CEACAM-1-/- recipients of WT T cells, and WT recipients of WT T cells. We conclude that CEACAM-1 deficiency on donor T cells or in recipients results in increased gut and systemic GVHD due to increased T cell activation and expression of the gut homing integrin α4β7. This suggests that the use of CEACAM-1 agonists could be a novel therapeutic strategy for ameliorating acute intestinal and systemic graft-versus-host-disease. Studies with an agonistic CEACAM-1 antibody are currently under way.
without clinically significant GVHD, we previously identified a panel of CML-associated antigens that are targets of antibodies present in post-DLI sera. To gain insight into the possible mechanisms of immunogenicity of our GvL-associated target antigens, we surveyed previously known cellular functions of our entire panel of identified antigens, and observed that ~70% are nucleic acid binding proteins, thus predicting that sera from DLI responders may contain nucleic-acid containing activities that stimulate immunity. Indeed, 5 of 6 post-DLI sera, but not pre-DLI sera, induced a 3 to 50-fold increase in the expression of MIP-1alpha, TNF-alpha, IP-10 and IFN-alpha transcripts in normal peripheral blood mononuclear cells (PBMC). Cytokines were also not induced by sera from 3 of 3 DLI non-responders or 3 of 3 CML patients who achieved molecular remission after imatinib treatment. This endogenous immunostimulatory factor required both nucleic acid and protein for its adjuvant activity as pretreatment of post-DLI sera with DNase, RNase, papain or pepsin resulted in marked decrease in cytokine induction. To test for the role of distinct TLRs in this response, HEK transfectants expressing TLR3, 4, 8 or 9 were exposed to sera collected 6-12 months after DLI. IL-8 expression increased only in the TLR8 and TLR9-expressing cell lines that are known to be responsive to RNA and DNA respectively. IL-8 expression was further induced by post-DLI sera in TLR9-expressing cell lines co-transfected with CD32, suggesting that internalization by FcR may enhance delivery of nucleic acids to endosomal TLRs.1 None of the transfectants could be activated by pre-DLI sera, nor by post-DLI sera from non-responders. Finally, sera from responders collected within 2 weeks after DLI consistently activated TLR8 and suggested that endogenous TLR8/9 activation may contribute to the early immunologic events involved in effective DLI responses. Taken together, these studies demonstrate for the first time that effective tumor immunity is correlated with the presence of endogenous adjuvant-like nucleic-acid-immunoglobulin complexes in patient sera. Ongoing studies are focused on studying the immunologic effects of this endogenous adjuvant and determining which of our previously identified CML antigens are bound to nucleic acids that activate TLR8 and TLR9.

**333 CYTOTOXIC T LYMPHOCYTE RESPONSES TO PRI PEPTIDE IN CML PATIENTS INVERSELY CORRELATE WITH PROTEINASE 3 AND ELASTASE EXPRESSION IN HLA-IDENTICAL DONOR PHENOTYPIC RESPONSES DETERMINED BY T-CELL ACTIVITY AND DETERMINATION OF A CYTOKINE INDUCTION WHILE SPARING GRAFT-VERSUS-LEUKEMIA ACTIVITY**

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The primary granule proteins (PGP) neutrophil elastase (ELA2) and proteinase 3 (PR3) both contain the nonapeptide PR1 which can induce cytotoxic T lymphocyte (CTL) responses in chronic myeloid leukemia (CML). The relative contribution of PR3 and ELA2 to PRI expression is not known. We previously found that higher levels of PR3 and ELA2 gene expression in CD34+ progenitor cells were associated with longer survival in CML patients. Eradication of leukemia requires elimination of leukemic stem cells, which reside within the CD34+ progenitor cell pool. We therefore studied PGP expression and T cell response to PRI in 31 CML patients and their HLA-identical family donors prior to T-cell depleted allogeneic stem cell transplantation (SCT) with T-cell add-back on day 45-100 post-SCT. CD8+ T cells and CD34+ progenitor cells were purified from mononuclear cells (MNC) of cryopreserved leukapheresis products from HLA-A,-B,-C matched donors and their progeny (MNC) gene expression in MNC and CD34+ cells was measured using real-time quantitative polymerase chain reaction (RQ-PCR). To assess PRI-CTL responses, T2 cells were loaded with PRI peptide (V10QELNNTV) and subsequently co-cultured with CD8+ T cells. PRI-CTL response was measured as interferon-γ mRNA expression (CD8+) obtained by RQ-PCR. PRI-specific CTL responses were detected in 7/28 CML patients and 7/27 HLA-identical donors. In CML patients, pre-SCT expression of both PR3 and ELA2 in MNC was strongly correlated with the expression in CD34+ cells (p=0.02 and p=0.01 respectively). There was an inverse relationship between PRI-CTL response in CML patients pre-SCT and PR3 or ELA2 expression (p=0.02 and p=0.01 respectively). This data suggest that both PR3 and ELA2 expression in CD34+ cells and their progeny are a potential source of PRI. However, high expression of these proteins may result in selective deletion of PRI T cell clones. The presence of PRI responses in HLA-identical donors was associated with a significantly improved molecular remission rate from 3 months post-SCT onwards, and moreover, was predictive of molecular remission at 1-year post-SCT (p=0.003). These findings support peri-transplant vaccination strategies with PRI peptide to eradicate minimal residual disease. Conversely, in untransplanted patients, the lower PRI response in the presence of high PGP expression suggests that vaccines given to patients with minimal residual disease could result in tolerance to the leukemia.

**334 CD18 SEPARATES GVHD AND GVL EFFECT**

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Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation. Migration of donor-derived T cells into GVHD target organs plays an essential role in the development of GVHD. Beta2 integrins, a group of heterodimeric molecules including CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), CD11c/CD18 and CD11d/CD18, are of critical importance for leukocyte extravasation through vascular endothelia and for T cell activation. We asked whether CD18-deficient T cells would induce less GVHD while sparing graft-versus-leukemia (GVL) effect. In murine allogeneic bone marrow transplantation (BMT) models, we found that recipients of CD18/- donor T cells had significantly less GVHD morbidity and mortality compared with recipients of WT donor T cells. A cell-dose titration experiment indicated that the ability of CD18-/ T cells had more than 4-fold reduction compared to that of WT T cells in the induction of GVHD. Analysis of alloreactive showed that CD18/- and WT T cells had comparable activation, expansion and cytokine production in vivo. Reduced GVHD associated with a significant decrease in donor-T cell infiltration of recipient intestine and with an overall decrease in pathologic scores in intestine and liver. Finally, we found that in vivo GVL effect of CD18-/ donor T cells was largely preserved, because mortality of the recipients transplanted with CD18-/ T cells plus tumor cells was greatly delayed or prevented. Our data suggest that strategies to target beta2 integrin have clinical potential to alleviate or prevent GVHD while sparing GVL activity.

**335 INHIBITION OF HMG-COA REDUCTASE ACTIVITY WITH STATINS PROVIDES ACUTE-GRAFT-VERSUS-HOST DISEASE PROTECTION BY TH-2 CYTOKINE INDUCTION WHILE SPARING GRAFT-VERSUS-LEUKEMIA ACTIVITY**

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The beneficial impact of 3-hydroxy-3-methyl-Coa reductase inhibitors (statins) on autoimmune and allograft rejection is well documented. To investigate whether statins are capable of protecting from acute graft vs host disease (aGVHD) we utilized an established murine model (FVB/N into Balb/c). Type II stasins were potent inducers of aGVHD and ELA2 and their T-helper cell (Th)2 cytokine profile in the adoptively transferred T cells upon exposure in vitro or in vivo. Expansion of alloreactive luciferase transgenic T cells as measured by total body light emission was significantly reduced by donor pre-treatment (10mg/kg) or in vitro T cell treatment (10nM) with atorvastatin (AT vs PBS; p=0.0008). The beneficial effect of statin treatment translated into significantly reduced aGVHD lethality in statin as compared to PBS treated animals. Th-2 biased donor T cells could be tracked until day 25 after BMT. Host treatment...