proliferated beyond 5th and 6th cell-divisions respectively, whereas the lovastatin treatment reduced the number to 31% and 48% respectively. In summary, we demonstrated here that lovastatin prevents both homing and proliferating of donor-derived T cells in the secondary lymphoid organs, which are crucial sites for alloreactive expansion. While most of the control mice died of acute GVHD within the first week of post-transplant when alloreactive T cells infiltrated the targeted organs, lovastatin treatment prevented the activation and expansion of donor-derived T cells, and thus reduced the GVHD mortality and morbidity. Our study provides rationale for a potential novel treatment for GVHD.

310

EXPRESSION OF STAT1 DURING GRAFT-VERSUS-HOST DISEASE (GVHD)

Ziegler, J.A.[1], Lokshin, A.[2], Bedeir, A.[3], Sepulveda, A.[3], Lentzsch, S.[1], Mapara, M.Y.[1] [1]University of Pittsburgh Cancer Institute, Pittsburgh, PA; [2]University of Pittsburgh,Luminex Facility, Pittsburgh, PA; [3]University of Pittsburgh, Department of Pathology, Pittsburgh, PA.

The role of the IFN-g in the development of GVHD is enigmatic due to an abundance of partially contradicting results. Whereas there is evidence that GVHD can occur in the absence of IFN-g, it has been demonstrated in preclinical BMT models that IFN-g may accelerate or mitigate GVHD depending on the experimental setting. We have focused on the role of STAT1 in the development of GVHD, the major signaling pathway of IFN-g. We studied STAT1 and p-STAT1 (Tyr701) expression by immunohistochemistry in the GVHD target organs liver, small bowel and colon following induction of GVHD and correlated these findings with the presence of lamina propria (LP) lymphocytes, typical features of GVHD-induced tissue damage and expression of tissue cytokines/chemokines. GVHD was induced in the fully MHC mismatched BALB/c to B6 strain combination following lethal irradiation with 975 rad. As detected by western blots p-STAT1 expression became detectable on day +1 in the spleen and on days +3 in the liver, small bowel and colon. Compared to untreated controls immunohistochemical p-STAT1 staining became apparent on day +3 post-BMT in the small bowel and colon of syngeneic controls and GVHD animals. Whereas p-STAT1 expression was only transient in syngeneic controls, a further increase in p-STAT1 staining was observed in GVHD animals in the colon and small bowel on day +6. In the colon this significant increase in crypt cell p-STAT1 staining was associated with the presence of LP infiltrating lymphocytes and coincided with the maximal features of tissue damage (luminal sloughing, crypt destruction and crypt apoptosis). In line with these results IFN-g protein expression became detectable in colon tissue lysates on day +6 supporting the role of IFN-g producing infiltrating donor T cells in causing STAT1 activation and tissue damage. We conclude that in comparison to untreated controls STAT1 activation can be observed in the colon and small bowel starting on day +3 in animals with GVHD and syngeneic controls. Whereas pSTAT1 staining peaks at day +3 in syngeneic controls and declines thereafter, maximal STAT1 activation occurs in GVHD animals on day +6 , coincides with detectable IFN-g expression and is accompanied by LP infiltration and features of severe GVHD-related tissue damage. To fully understand the role of IFN-g in the development of gut GVHD further studies are warranted to delineate the role of STAT1 dependent and independent signaling pathways in the development of GVHD.

311

THE PREDICTIVE VALUE OF GENE EXPRESSION PROFILES FOR ACUTE GRAFT-VERSUS-HOST DISEASE AFTER HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING FOR HEMATOLOGICAL MALIGNANCY

Masmas, T.N.[1], Friis-Hansen, L.[2], Petersen, S.L.[1], Schjerling, C.K.[2], Hansen, F.C.[2], Vindelov, L.L.[1] [1]Allogeneic Hematopoietic Cell Transplantation Laboratory, Department of Hematology, Rigshospitalet, Copenhagen, Denmark; [2]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark.

Purpose: To test the hypothesis that global gene expression profiles of peripheral blood mononuclear cells (PBMNC) day +14 after hematopoietic cell transplantation (HCT) with nonmyeloablative conditioning could predict the later occurrence of acute graft-versus-host disease (aGVHD) grade II-IV. **Material:** Ninety-eight patients with hematological malignancies received HCT with peripheral blood stem cells from an HLA-identical sibling/mother donor (N=64/1) or from a matched unrelated donor (N=33) following nonmyeloablative conditioning with low dose fludarabine and 2 Gy of total body irradiation. Post-transplant immunosuppression consisted of cyclosporine and mycophenolate mofetil. Among these patients, 16 patients never experiencing aGVHD and 16 patients experiencing aGVHD grade II-IV before day +70 (range 21-70) were selected. **Methods:** RNA was precipitated from frozen PBMNC from day +14 post-transplant and gene profiling analyses were performed using Human Genome U133 Plus 2.0 GeneChip Array. The array data were normalized and GCMA modelled in R, log2 transformed, corrected for batch variation, and subsequently imported into dChip for further analysis. **Results:** The diffentially regulated gene expression between the two groups was identified and formed the basis for the subsequent principal component analysis. This separated more than 85% of patients who experienced aGvHD from those who did not. **Conclusion:** Albeit preliminary, these data suggest that pattern of gene expression profiles early post-transplant seems to be able to predict patients with high risk of later occurrence of aGVHD from those never experiencing aGVHD in this retrospective study. This knowledge could be exploited to increase the immunosupression and thus prevent aGVHD in patients at risk. Furthermore, this method could help identify candidate genes of interest for the pathogenesis of aGVHD.

312

DENDRITIC CELL TYPE 2 COUNTS ON DAY 28 IN HLA-MATCHED RELATED ALLOGENEIC PBSCT PREDICTS THE INCIDENCE OF ACUTE AND CHRONIC GVHD

Rajasekar, R.[1], Mathews, V.[1], Lakshmi, K.M.[1], Viswabandya, A.[1], George, B.[1], Chandy, M.[1], Srivastava, A.[1] [1]Christian Medical College, Vellore, Tamil Nadu, India.

Dendritic cells (DC) are antigen-presenting cells involved in induction and regulation of immune responses. We investigated the impact of the number of infused and engrafted (day 28) dendritic cells, DC1 ($lin^-HLA\text{-}DR^+CD11c^+$) and DC2 ($lin^-HLA\text{-}DR^+CD123^+$), on the development of acute and chronic GVHD. 68 patients who underwent HLA matched related G-CSF mobilized allogeneic PBSCT were included in the analysis. The median age was 28 years (range: 3-55) and there were 43 (63%) males. Conditioning regimen was myeloablative in 34 (Bu/Cy=20; Cy/TBI = 14) and reduced intensity in the rest (Flu/Mel =12; Flu/Cy =15; Flu/Cy/ATG =3; Flu/Bu/Cy=1; Flu=1; Ida/Flu/Cytosine=2). All patients received cyclosporine and short course methotrexate as GVHD prophylaxis. 23 patients developed acute GVHD (grade II-IV) and 21 patients had chronic GVHD. Twelve patients received steroids before day 28 for treatment of GVHD. Seven patients died before day 28 and were excluded from the analysis; 2 of these patients had acute GVHD. On a univariate analysis day-28 total DC, DC1 and DC2 were significantly associated with development of acute and chronic GVHD while graft total DC, DC1 and DC2 did not show a similar association. Using a ROC curve plot analysis, cutoff values for day-28 DC (Total DC=10.7/ul, DC1=9.7/ul and DC2 = 4.5/ul) gave the highest likelihood ratios for acute GVHD (2.77, 2.14and 3.29 respectively). These cut off values significantly discriminated patients probability of developing acute and chronic GVHD on a univariate analysis. On a multivariate analysis, a low day-28 DC2 (≤4.5/ul) together with patient age retained their risk for acute GVHD (HR=67.74 and 1.05, P-values 0.000 and 0.042 respectively), while for chronic GVHD only a low day-28 DC2 remained significant (HR=12.8, P=0.005). Using the DC2 cutoff value of 4.5/ul, patients were categorized into a high (> 4.5/ul) (n=31) and a low DC2 (≤4.5/ul) (n=30) group. These two groups were comparable with regard to age, sex, F>M, conditioning regimen and graft