5,10-methylenetetrahydrofolate reductase (MTHFR) is a central regulatory enzyme in folate metabolism. A common functional polymorphism of the MTHFR gene occurs at C677T. The 677TT genotype produces an enzyme with only 30% of the activity of the wild-type (677CC) enzyme. The *MTHFR* polymorphism has been shown to affect the sensitivity of patients to folate-based drugs such as methotrexate (MTX) that is used for GVHD prophylaxis following allogeneic hematopoietic stem cell transplantation (HSCT). To assess the significance of C677T genotypes in HSCT using MTX as a GVHD prophylaxis, we analyzed DNA from 159 patients with a hematological disease and their HLA-identical sibling donors using PCR-RFLP method. The frequencies of CC, CT and TT genotypes in the patients were 35%, 52% and 13%, respectively, and in the donors were 30%, 62% and 8%, respectively. There was no significant difference in the distribution patterns of the C677T genotypes between patients and donors ($P = 0.19$). Multivariate analysis revealed a significantly lower incidence of grade I-IV acute GVHD in patients with the 677TT genotype (relative risk, 0.35; 95% confidence interval, 0.13–0.95; $P = 0.040$) and a non-malignant disease (0.22; 0.05–0.89; 0.034). There was a significant association between a lower incidence of grade II-IV acute GVHD and the use of bone marrow for transplantation (0.32; 0.11–0.91; 0.032). There was no association between the incidence of acute GVHD and the donor C677T genotypes. We analyzed the incidence of acute GVHD in relation to the *MTHFR* genotype using the Kaplan-Meier method. The incidence of grade I-IV acute GVHD in the patients with 677TT genotype was significantly lower than in those with 677CC/CT genotype (19% versus 45%, $P = 0.035$). There was a trend for a lower incidence of grade II-IV acute GVHD in patients with 677TT genotype compared with 677CC/CT genotype (5% versus 24%, $P = 0.077$). The C677T genotypes in the patient or donor were not associated with the treatment-related mortality, relapse rate, relapse-free survival and overall survival. These results suggest that greater immunosuppression by MTX due to low MTHFR enzyme activity decreases the risk of acute GVHD in recipients of allogeneic HSCT. Further studies are needed to confirm that the *MTHFR* C677T polymorphism can predict the outcome of HSCT using prophylactic MTX to prevent GVHD.

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HOMEOSTATIC AND INFLAMMATORY PROCESSES CONTRIBUTE TO ELEVATED PLASMA LEVELS OF B CELL ACTIVATING FACTOR (BAFF) IN CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD)

Hakim, F.T., Rehman, N., Dickinson, J., Beal, M., Baskar, S., Rader, C., Cowen, E., Pavletic, S., Gress, R.E. National Cancer Institute, NIH, Bethesda, MD.

BAFF, a non-redundant cytokine produced by myeloid cells, plays a critical role in the normal homeostatic maintenance, activation and function of B cells. Elevated circulating levels of BAFF, however, have been observed in systemic autoimmune disorders and, in murine models, have been linked to a failure to delete auto-reactive B cells. We similarly observed elevated plasma BAFF levels in 77 patients in an ongoing NCI CGVHD natural history protocol, with a median of 2845 pg/ml (range 92 to 17058), as compared to 556 pg/ml (range 75 to 1834) in 18 normal donors. Furthermore, in a subset of 22 patients in which severity of CGVHD could be assessed by the presence of marked erythema or sclerosis on 10 to 90% of their body surface areas (BSA), the BAFF level correlated with the percentage of affected BSA (Spearman r = +.63). We then explored the factors that might contribute to elevated BAFF levels. In recipients recovering from either autologous or allogeneic transplant (without GVHD) we observed the highest BAFF levels at day 0 (median of 10534 and 12240 pg/ml respectively), when B cells were severely depleted. As B cell populations recovered to normal levels post transplant, plasma BAFF concentrations declined (Spearman r = -.80 and r = -.60, respectively), consistent with homeostatic cytokine-consumption dynamics. Despite comparably high levels of BAFF (median of 11342 pg/ml) at transplant day 0 in 16 patients who later developed CGHVD, BAFF levels in the cross-sectional, natural history patient population were only moderately correlated with the degree of post transplant B cell recovery (r = -.48). Since inflammatory triggers can induce elevated BAFF production by dendritic cells, we assessed plasma levels of cytokines indicative of an inflammatory process. In 34 patients, the plasma levels of IP-10 and sTNFRII correlated positively with BAFF levels (r = +.627 and r = +.642, respectively), consistent with active inflammatory processes in those CGVHD patients with elevated BAFF levels. In a multi-step regression model, the levels of circulating B cells, plasma IP-10 and sTNFRII combined to strongly predict BAFF levels (R = .834). These findings suggest that both homeostatic recovery of B cell populations consuming BAFF and inflammatory cytokine cascades initiated by donor-anti-host reactivity combine to regulate BAFF levels post transplant.

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CORRELATION BETWEEN FOXP3 GENE POLYMORPHISMS IN DONORS, AND THE SEVERITY OF ACUTE GRAFT-VERSUS-HOST DISEASE IN PATIENTS AFTER RELATED ALLOGENEIC STEM CELL TRANSPLANTATION

Perfecto-Avalos, Y.[1], Borbolla Escoboza, J.R.[1], Villela-Martiez, L.M.[1], Scott, S.P.[1], Vela-Ojeda, J.[2], Gonzalez-Ramella, O.[3], Baltazar-Arellano, S.[4], Lopez-Hernandez, M.A.[5]. [1] ITESM Medical School, Monterrey, Nuevo Leon, Mexico; [2] Centro Medico La Raza, IMSS, Mexico City, DF, Mexico; [3] Hospital Civil de Guadalajara "Juan I Menchaca", Guadalajara, Jalisco, Mexico; [4] HUMAE 25, IMSS, Monterrey, Nuevo Leon, Mexico; [5] Centro Medico 20 de Noviembre, Mexico City, DF, Mexico.

Introduction: Acute Graft-versus-host disease (aGVHD) is a major complication of allogeneic stem cell transplantation (alloSCT). Risk factors include patient age, sex matching, CMV status and degree of match. Regulatory T cells are critical for immune tolerance processes such as aGVHD, and express the transcription factor FOXP3, a member of the forkhead/winged-helix family, identified as a key regulatory gene required for the development and activity of these cells. It has been suggested that genetic expression of FOXP3 is inversely correlated with the severity of the GVHD. We studied donors DNA looking for 5 polymorphisms on the promoter region of the FOXP3 gene, and we tried to correlate them with presence and degree of aGVHD. **Patients and Methods:** We studied donors of stem cells for allogeneic stem cell transplants. We looked for the presence of the following polymorphisms by PCR: POL01–5906 T/A rs2869211; POL03–3279 A/C rs3761548; POL04–2383 C/T rs3761549; POL05–1383 C/T rs2232364; POL06–924 A/G rs2232365. **Results:** Our sample consisted of 31 donors, all siblings. In them we found only 2 of the 5 FOXP3 polymorphisms, either as homozygous or heterozygous. These polymorphisms were found in 15/31 donors, with 12 being homozygous (38.7%), and 3 heterozygous (9.7%). These genes polymorphisms were POL03 y POL06. The most observed polymorphism was POL-06 with 9 cases, while POL-03 was found in 6 donors. Only sex difference and CMV status had an elevated hazard ratio for developing GVHD (HR = 1.18, CI95%: 0.18 to 7.64; p = 0.85) and (HR = 3.0, CI95%: 0.07 to 126; p = 0.46) respectively. We found no statistically significant difference in the incidence of GVHD between patients who had received cells from donor with or without a FOXP3 polymorphism (p = 0.87). When we analyzed the risk of presenting GVHD, results suggest that having one of the 2 positive polymorphisms of the FOXP3 gene could have a protective effect for the patient. For POL03 HR = 0.87, CI95%:0.18 to 4.14; p = 0.86, and for POL06, HR = 1, CI95%: 0.37 to 2.64, p = 0.67. **Conclusions:** Even though our sample is still small to make conclusive remarks, we believe that our results point toward some level of protection from acute GVHD in patients receiving cells from donors expressing POL03 and POL06. It is also worthwhile mentioning the relatively high frequency of these polymorphisms, as well as the absence of the other 3.

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HUMAN NEUTROPHIL ELASTASE STIMULATING CD4+ AND CD8+ T CELLS IS A POTENTIAL PROTEIN VACCINE FOR LEUKEMIA PATIENTS WITH DIVERSE HLA TYPES

Le, Q., Melenhorst, J.J., Hensel, N., Eniafe, R., Barrett, A.J. National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

The detection of naturally occurring T cells responding to proteins overexpressed in myeloid leukemia suggests that vaccines could boost the immune response to leukemia and achieve disease control. Human neutrophil elastase (HNE) is a myeloid-restricted protein highly expressed in myeloid leukemia cells. HNE protein can induce both CD4+ and CD8+ T cell responses in healthy individuals including HLA-A*0201 negative individuals. To develop HNE protein vaccine, and to identify major histocompatibility complex (MHC) class II restricted or HLA-A*0201 negative MHC class I restricted epitopes in HNE, we stimulated peripherial blood mononuclear cells (PBMC) from 6 myeloid leukemia pre-transplant patients with purified full length HNE protein. HNE protein induced a response in CD4+ and CD8+ T cells from 4 patients with acute myeloid leukemia (AML), as measured by IFN-γ protein expression in flow cytometric assay. However, no response was detected in CD4+ and CD8+ T cells from 2 pre-transplant patients with chronic myeloid leukemia (CML). Responses of CD4+ and CD8+ T cells to HNE protein stimulation were enhanced by HNE expressing antigen-presenting T cells (T-APC), as measured by flow cytometric assay. HNE protein induced significant IFN-γ expression in CD4+ and CD8+ T cells at 540X and 10X above background in an (HLA-A11, 31) AML patient (patient #1), and at 6X and 3.5X above background in an (HLA-A*0205, 03*) AML patient (patient #2), as measured by quantitative Real-Time RT-PCR assay (QRT-PCR). HNE protein also induced significant IL-2 production in CD4+ and CD8+ T cells at 7.7X and 6.1X above background in patient #1. Finally, we showed by QRT-PCR that the T cell responses to HNE protein stimulation were associated with the levels of HNE gene transcripts in the PBMC. Further inverstigation of the correlation between HNE protein specific CD4+ and CD8+T-cell responses and HNE gene transcripts in consecutive samples obtained pre- and post-SCT may define an ideal time window to vaccinate post-SCT patients with myeloid leukemias. We found that full-length HNE protein stimulated both CD4+ and CD8+ T cell responses in HLA-A*0201 negative patients with AML in the first three months following SCT. These results provide the rationale for using HNE protein to vaccinate patients with myeloid leukemias after SCT to improve graft-versus-leukemia effects.

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ETHYLENECARBODIIMIDE (ECDI) COUPLED ALLOGENEIC ANTIGEN PRESENTING CELLS INDUCE HUMAN CD4+ REGULATORY T CELLS

Magg, T.[1], Yu, X.-Z.[2], Albert, M.H.[1]. [1] Dr. von Haunersches Kinderspital der LMU, Munich, Germany; [2] H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

Adoptive transfer of polyclonal CD4+CD25+ regulatory T cells (nTregs) can tolerize transplantation alloresponses and prevent lethal acute graft-versus-host disease (GVHD) in mice. However isolation of human CD4+CD25+ nTregs for adoptive immunotherapy in sufficient numbers is cumbersome and prone to contamination with non-regulatory, alloreactive CD25+ T-cells. Incubation of ethylenecarbodiimide (ECDI)-coupled antigen presenting cells (APC) with naïve T-cells and antigen has been shown to induce tolerance in various experimental models.

We have therefore hypothesized that ECDI-coupled APC would be able to tolerize alloreactive naïve human CD4+ T-cells from HLA-incompatible human donors, and have tested whether this would induce a Treg-like CD4+ population (ECDI-Tregs). We further investigated whether these cells could be expanded ex vivo without loss of their regulatory capacity.

After 5 days of culture, ECDI-Tregs were as potent as freshly isolated nTregs to suppress proliferation of effector T-cells in a mixed lymphocyte reaction with allo-APC from the original APC donor and they maintained high expression of CD62L and CD27 as well as low CD127 expression. Foxp3 mRNA levels and Foxp3 protein expression was significantly increased in ECDI-Tregs compared to cells cultured with untreated APC or freshly isolated CD4+ cells, indicating either de novo induction or preferential expansion of Tregs. ECDI treatment of APC resulted in increased apoptosis and downregulation of costimulatory molecules CD40, CD80 and CD86 while HLA-DR expression remained unchanged compared to untreated cells. This might implicate T-cell receptor - MHC interaction without proper costimulation as a possible mechanism for ECDI-Tregs induction. Addition of IL-2 and rapamycin and weekly re-stimulation with untreated allo-APC led to exponential expansion of ECDI-Tregs with increasing foxp3 levels and without loss of their suppressive activity after 28 days.

These findings provide a proof of principle that ECDI-coupled allo-APC can induce a potent regulatory T-cell population that can be expanded ex vivo. These inducible Tregs suggests a novel approach to enhance the feasibility and effectiveness of inducing tolerance by Tregs as an adoptive immunotherapy in transplantation.

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MACROPHAGE MIGRATION INHIBITORY FACTOR AS A NEW TARGET IN GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Chang, Y.-Y.[1], Miklos, S.[1], Mueller, G.[1], Holler, E.[1], Lindner, P.[2], Leng, L.[3], Schubert, T.[4], Bucala, R.[3], Hildebrandt, G.C.[1]. [1] University of Regensburg Medical Center, Regensburg, Germany; [2] University of Regensburg Medical Center, Regensburg, Germany; [3] Yale University School of Medicine, New Haven, CT; [4] Institute for Pathology, Frankfurt, Germany.

Acute graft versus host disease (GVHD) is the major complication after allogeneic (allo) stem cell transplantation (SCT) involving injury to host tissues by both inflammatory cytokines and donor-derived cellular effectors. Macrophage migration inhibitory factor (MIF) is produced by various cell types and has a broad range of proinflammatory properties. We tested, whether MIF contributes to GVHD by using a well established murine model. Lethally (1300 cGy) irradiated B6D2F1 (H-2bxd) mice received SCT either from syngeneic (syn) (B6D2F1) or allo (B6; H-2b) donors. One half of animals after either syn or allo SCT were treated with polyclonal antibodies against mouse MIF from day 0 until day 14, whereas the other halves of syn and allo recipients received control IgG. GVHD severity was assessed after SCT by survival and a clinical scoring system assessing weight changes, fur texture, skin integrity, mobility and posture. Syn recipients stayed clinically well and all animals survived regardless their treatment with control or anti-MIF. Animals receiving allo-SCT plus control IgG developed significant aGVHD and high mortality. By contrast, in allo anti-MIF treated recipients, GVHD scores (day 21: 4.4 ± 0.4 vs. 5.7 ± 0.5) and weight loss (24.6% vs. 45.4%) were reduced, and survival significantly improved ($p < 0.05$). In addition, target organ injury to the gut (histopathology score: 4.3 ± 0.7 vs. 8.0 ± 2.6) as well as serum IFNγ (4397 ± 995 vs. 6070 ± 525 pg/ml) and TNFα (113.9 ± 12.7 vs. 159.2 ± 22.5 pg/ml) levels of anti-MIF treated allo recipients were decreased by day 7 when compared to controls. Alloantigen-specific T cell activation in vitro was significantly suppressed in the presence of anti-MIF, as determined by a reduction in T cell proliferation, IFNγ and TNFα secretion.

We next challenged syn and allo recipients with P815 lymphoblast-like mastocytoma cells (H-2d) at the time of transplantation to asses the effect of anti-MIF on graft versus leukemia (GVL) responses. Significant P815 cell infiltration was seen after syn SCT as demonstrated by FACS analysis of the spleens (67.2% infiltration) and by histopathology of the liver between day 14 and day 21 after SCT. In contrast, P815 cells were significantly cleared in allo recipients treated with either control or anti-MIF (0.81% and 12.1% infiltration).

In summary, our data show an important role for MIF in GVHD pathophysiology and suggest MIF as a promising target in reducing GVHD without the loss of GVL.

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CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD) FOLLOWING SIBLING DONOR PERIPHERAL BLOOD STEM CELL TRANSPLANT (PBSCT) VERSUS BONE MARROW TRANSPLANT (BMT): GREATER INCIDENCE AND LONGER DURATION OF IMMUNE-SUPPRESSION (DIS) USING PBSC

Arora, M., Nagaraj, S., DeFor, T.E., MacMillan, M.L., Wagner, J.E., Burns, L.J., Weisdorf, D.J. University of Minnesota, Minneapolis, MN.