PR1 is also processed and presented by NE, which may result in enhanced immunogenicity of the peptide compared to peptides derived from a single protein. Redundancy of peptides may also lessen the impact of tumor-loss variants after PR1-based immunotherapy.

93 TREATMENT OF DONORS WITH INTERLEUKIN-18 REDUCES ACUTE GRAFT-VERSUS-HOST DISEASE VIA STAT6 AND PRESERVES CD8+ MEDIATED GRAFT-VERSUS-LEUKEMIA EFFECTS


Interleukin 18 modulates both Th1/Th2 responses and stimulates hematopoietic growth factor secretion. We investigated the effect of pretreating bone marrow transplant (BMT) donors with IL-18 on the severity of acute GVHD using a well-characterized irradiated experimental BMT model (BALB/c(H-2d) to B6 (H-2b). Pretreatment of allogeneic BMT donors with IL-18 (1µg/day of rmIL-18 for 10 days, day -11 to -1) significantly improved survival, and caused significant reduction in serum levels of TnFα (21.1±19pg/ml vs 524±69pg/ml, P<0.05) and LPS (5.3±1.1U/ml vs 12.4±1.3U/ml, P<0.05) and GVHD related intestinal histopathology (P<0.05). All surviving mice showed complete donor engraftment, thus ruling out graft rejection and mixed chimeras as causes for decreased GVHD. IL-18 pretreatment was also associated with reduced IFN-γ (1987±387pg/ml vs 4606±94pg/ml, P<0.03) and greater IL-4 secretion (54.8±3.9pg/ml vs 14.5±3.4pg/ml, P<0.04) by donor T cells after BMT. Surprisingly, when IFN-γ deficient mice were pretreated with rmIL-18 and used as donors, acute GVHD mortality was reduced thus ruling out the role of donor IFN-γ in this effect. Acute GVHD mortality was also reduced when IL-18 was administered to donors deficient in STAT4. Treatment of STAT6 deficient donors with IL-18 did not alter IFN-γ secretion (14.3±2.1ng/ml vs 10.2±3.8ng/ml, P=NS) or enhance IL-4 production. Furthermore pretreatment of STAT1-/- donors with IL-18 did not reduce acute GVHD mortality thus confirming the role of STAT6 signaling in IL-18's protective effect.

94 TACROLIMUS (FK-506) AND MYCOPHENOLATE MOFETIL (MMF) GVHD PROPHYLAXIS IN ALLOGENIC SCT (ALLOSCC) RECIPIENTS: ALTERED MMF PHARMACOKINETICS (PK) ASSOCIATED WITH AGVHD

ALTERED MMF PHARMACOKINETICS (PK) ASSOCIATED WITH AGVHD

Rini BI et al, with IL-18 did not reduce acute GVHD mortality thus confirming that it was due to antibody dependent cellular cytotoxicity (ADCC) of CD83+ cells. In additional experiments we showed that activated NK-cells lye 5+ Cr labelled CD83+ MoDC in the presence of non-immune polyclonal antibody. Immature CD83+ MoDC were not lysed in the presence of RA83. However, lysis of MoDC did not account for all the effect because stimulation with allogeneic MoDC fixed with paraformaldehyde also exhibited inhibition of T lymphocyte proliferation in the presence of RA83. This implied that RA83 mediated immunosuppression that activated blasting T cells (CD25+, Ki67+) stimulated by allogeneic MoDC fixed with paraformaldehydelye also exhibited inhibition of T lymphocyte proliferation in the presence of RA83. This implied that CD83+ targets of ADCC must exist or arise in the responder cell preparation. We then established that activated blasting T-cells (CD25+, Ki67+) stimulated by allogeneic MoDC also express CD83 and that they become targets of NK-cell mediated ADCC. Thus, CD83 is expressed by both stimulator and responder cells in the MLR. Simultaneous targeting of both activated cell populations by a single reagent such as anti-CD83 may be a potent means of achieving therapeutic immunosuppression for allogeneic hematopoietic stem cell transplantation.

95 CD83 ANTIGEN: A POTENTIAL NEW TARGET FOR GRAFT VERSUS HOST DISEASE PREVENTION

Minnety, D.J.; Kato, M.; MacDonald, K.P.; Rice, A.M.; Hart, D.N. Cancer Biotherapy, Mater Medical Research Institute, South Brisbane, QLD, Australia.

Novel strategies are required to overcome the limitations imposed on hematopoietic stem cell transplantation by graft versus host disease (GVHD). Current GVHD therapies focus on elimination or attenuation of the effector T cell. There is emerging evidence in the literature to suggest a primary role for dendritic cells (DC) in the initiation of GVHD. CD83 is a marker of DC differentiation/activation. Polyclonal anti-CD83 antibody (RA83) inhibits the T lymphocyte proliferative response in the mixed leucocyte reaction (MLR) stimulated by allogeneic monocyte derived DC (MoDC). We investigated the mechanism of this RA83 mediated immunosuppression and found that it was not due to functional blockade of CD83. It required the presence of NK-cells. Fab fragments of RA83 did not inhibit the MLR, and treatment with an Fc plug receptor III (CD16) blocking monoclonal antibody (3G8) prevented the blockade, indicating that it was due to antibody dependent cellular cytotoxicity (ADCC) of CD83+ cells. In additional experiments we showed that activated NK-cells lye 5+ Cr labelled CD83+ MoDC in the presence of non-immune polyclonal antibody. Immature CD83+ MoDC were not lysed in the presence of RA83. However, lysis of MoDC did not account for all the effect because stimulation with allogeneic MoDC fixed with paraformaldehydelye also exhibited inhibition of T lymphocyte proliferation in the presence of RA83. This implied that CD83+ targets of ADCC must exist or arise in the responder cell preparation. We then established that activated blasting T-cells (CD25+, Ki67+) stimulated by allogeneic MoDC also express CD83 and that they become targets of NK-cell mediated ADCC. Thus, CD83 is expressed by both stimulator and responder cells in the MLR. Simultaneous targeting of both activated cell populations by a single reagent such as anti-CD83 may be a potent means of achieving therapeutic immunosuppression for allogeneic hematopoietic stem cell transplantation.

96 IMPROVED OUTCOME IN ACUTE LETHAL MURINE GRAFT VERSUS HOST DISEASE (GVHD) FOLLOWING ADMINISTRATION OF THE PROTEASOME INHIBITOR PS-34I

Gatvin, ft.; Gatvin, ft.; Cooke, lV; FeJv'ara, J.LJ 1. Internal Medicine, University of Michigan, Ann Arbor, MI; 2. University of Florida, Gainesville, FL.

Proteasome inhibitors have recently shown much promise by demonstrating that proteasome inhibition is an effective therapeutic strategy for decreased GVHD. IL-18 pretreatment was also associated with reduced IFN-γ (1987±387pg/ml vs 4606±94pg/ml, P<0.03) and greater IL-4 secretion (54.8±3.9pg/ml vs 14.5±3.4pg/ml, P<0.04) by donor T cells after BMT. Surprisingly, when IFN-γ deficient mice were pretreated with rmIL-18 and used as donors, acute GVHD mortality was reduced thus ruling out the role of donor IFN-γ in this effect. Acute GVHD mortality was also reduced when IL-18 was administered to donors deficient in STAT4. Treatment of STAT6 deficient donors with IL-18 did not alter IFN-γ secretion (14.3±2.1ng/ml vs 10.2±3.8ng/ml, P=NS) or enhance IL-4 production. Furthermore pretreatment of STAT1-/- donors with IL-18 did not reduce acute GVHD mortality thus confirming the role of STAT6 signaling in IL-18’s protective effect.

96 IMPROVED OUTCOME IN ACUTE LETHAL MURINE GRAFT VERSUS HOST DISEASE (GVHD) FOLLOWING ADMINISTRATION OF THE PROTEASOME INHIBITOR PS-34I

Webiah, L.A.1; Webiah, L.A.1; &Webiah, K.2; Sayevs, T.J.2; Mzlrpby, I4~J. l I. Microbiolo-
Poster Presentations - Session I

97 TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML) RELAPSING AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION WITH STI-571 (IMATINIB MESYLATE) WITH OR WITHOUT DONOR LYMPHOID INFUSION (DLI)


CML relapsing after allogeneic stem cell transplantation (SCT) can be successfully retreated with DLI. However DLI can cause GVHD and is less effective for hematological relapse. We therefore used STI-571 to treat patients with CML relapsing after SCT following myeloablative preparative regimens. Ten patients (9 who had not received STI-571 pretransplant) relapsed 4-36 months post-transplant. Five were molecular relapses, five were more advanced (karyotypic relapse) achieved complete molecular remissions, sustained in three of I0 STI-treated patients, 7 achieved molecular remission which was sustained in 4. These results suggest that the cytoreductive action of STI can synergize with the graft-versus-leukemia effect. However, the tendency for further relapse when STI is withdrawn suggests that cure of relapsed CML may also require a GVL effect.

98 INFUSION OF HIGH NUMBERS OF G-CSF MOBILIZED BLOOD DENDRITIC CELLS TYPE 2 (DC-2) IS ASSOCIATED WITH AN INCREASED RATE OF CHRONIC GVHD IN ALLOGENEIC PBSC TRANSPLANTATION


It was previously shown that dendritic cells type-2 (DC-2) are significantly increased in G-CSF-mobilized leukapheresis products as compared to unstimulated bone marrow. In this study, we analysed whether the numbers of DC-1 and DC-2, as well as of other cell components in the graft, was associated with acute and/or chronic GVHD in 31 adult patients (11 MM, 7 CML-CP, 6 AML, 6 receiving NEI, and 2 MDS) receiving an allogenic PBSC transplant from HLA-matched siblings. Average cell doses (x106/kg) in the grafts were the following: 283±137 CD3+ T cells, 1658±884 CD4+ T lymphocytes, 1165±55 CD8+ T lymphocytes, 64±39 CD19+ B lymphocytes, 51±29 CD56+ NK cells, 253±103 CD14+ monocytes, 6.6±9.1 CD34+ cells, 2.1±0.9 HLA-DR+CD11c+ DC-1 and 2.9±1.3 HLA-DR+lin-CD123+ DC-2. Median follow-up was 255 days (range: 50-685). Patients were initially divided in three groups according to whether they had shown no signs of acute GVHD (grade 0, n=10), acute GVHD grade 1 (n=12), or grade II - IV (n=9). Median numbers of CD34+ cells, lymphocyte subsets, and CD-1 and DC-2 received by patients in these three groups did not differ significantly. Of 21 patients with adequate follow up (median 485 days, range: 131-695) 12 developed chronic GVHD (10 extensive, 2 limited). Analysis of cell components of the grafts demonstrated that patients developing chronic GVHD had received a significantly higher dose of DC-2 than patients without chronic GVHD (3.4±1.5 vs 2.2±0.9, p=0.05), while the dose of DC-1 (p=0.7), monocytes (p=0.28), T lymphocytes (p=0.643), B lymphocytes (p=0.939), NK cells (p=0.487) and CD34+ cells (p=0.757) was not different. Also, chronic GVHD did not correlate with recipient or donor age or gender, interval between diagnosis and transplantation, presence of acute or chronic conditioning regimen, or type of GVHD prophylaxis. Our results suggest that the presence of large DC-2 numbers in the graft may be associated with a higher risk of chronic GVHD after allogeneic PBSC transplantation. These data might prompt further studies addressing whether depletion of graft DC-2 might be beneficial in this setting.

99 SOLID TUMOR VACCINES ELICIT DISTINCT IMMUNE RESPONSES FROM HOST VERSUS DONOR T CELLS IN MIXED CHIMERAS CREATED WITH NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION (NST)

Durstovici, N.; Slansky, J.B.; Pardoll, D.M.; Fuchs, E.J.; Lukic, L. 1. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; 2. Dept. of Immunology, Univ. of Colorado Health Sciences Center, Denver, CO.

A growing body of evidence suggests that functional unresponsiveness of the immune system to growing solid tumors may be due to tolerance in tumor-specific CD4+ T cells, whereas tumor-specific CD8+ T cells exist in a state of partial activation with limited effector function. We therefore hypothesized that an alloimmune graft-versus-host reaction may provide a helper effect that augments the anti-tumor immune response of host CD8+ T cells in mixed hematopoietic chimeras. To test this hypothesis, we analyzed host versus donor T cell responses to AH1, an H-2Ld-restricted tumor antigen expressed by CT-26, a colon cancer of BALB/c mice. BALB/c mice with pre-established subcutaneous tumor underwent NST from MHCl-compatible B10.D2 donors. Two weeks later, groups of chimeras received nothing, B10.D2 splenocytes IV, CT-26 tumor vaccine (irradiated CT-26 cells mixed with a GM-CSF secreting bystander cell line), or both. By staining with H-2Ld tetramers loaded with the AH1 423-431 a.a. peptide, AH1-specific CD8+ T cells were found to comprise 2-3% of total spleen CD8+ T cells three weeks after vaccination. In contrast, AH1-specific T cells could not be detected in the non-transplanted, tumor bearing mice. To further characterize AH1-specific T cells we characterized T cell avidity for antigen, as measured by binding of AH1 peptide-loaded, L2 IgG dimers. AH1 specific T cells were expanded in vitro using AH1 peptide-transduced irradiated BALB/c splenocytes. After four weeks of stimulation, the highest percentages of cultures containing AH1-specific T cells were derived from tumor-bearing chimeras that received B10.D2 splenocytes and a CT-26 vaccine. Expanded cultures contained AH1-specific CD8+ T cells derived from the host and the donor, based on the differential expression of Ly9.1 marker on the