ASBMT Best Abstract Awards for Outstanding Basic Science Research

Each year the American Society for Blood and Marrow Transplantation presents Best Abstract Awards to recognize outstanding research in the basic sciences that contribute to the advancement of the field of blood and marrow transplantation. The abstracts receiving the award are those that were scored highest by the Abstract Review Committee. Each award is accompanied by a prize of $1,000. The awards are supported by an unrestricted educational grant from SuperGen, Inc.

1

THE ROLE OF PEYER'S PATCHES AND MESENTERIC LYMPH NODES IN ACUTE GVHD: A STUDY GUIDED BY BIOLUMINESCENCE IMAGING IN VIVO

Beilhack A.[1], Schulz S.[1], Wieland C.B.[1], Beilhack G.F.[1], Baker J.[1], Cao Y.-A.[2], Herman E.I.[1], Baker E.M.[1], Contag C.H.[2], Negrin, R.S.[1] 1. Dept. of Medicine, Div. of BMT Stanford University, Stanford, CA; 2. Dept. of Pediatrics, Stanford University, Stanford, CA.

Acute graft versus host disease (aGVHD) represents one of the major obstacles in allogeneic bone marrow transplantation. Our aim was to precisely determine the spatiotemporal evolution of aGVHD in vivo, with special regard to the role of mucosa associated lymphatic tissues, particularly Peyer's patches (PP), and mesenteric lymph nodes (mLN).

To visualize immune responses in living animals we developed and characterized a luciferase expressing (luc+) transgenic mouse line (FVB/N, H-2K^{q}). Bioluminescence imaging (BLI) and luciferase assays on purified transgenic donor cell populations revealed luciferase expression in T, B, NK cells, granulocytes and macrophages. Recipient mice (Balb/c, H-2K^{d}) were total body irradiated (800rads) and subsequently transplanted with 4×10^{6} luc+ splenocytes (Splc).

We detected the first bioluminescent signals in PP and mLN within 12 hours after transfer of luc+ allogeneic Splc. Using triple-color immunofluorescence microscopy (IFM), we could show that the majority of infiltrating donor cells in PP consisted of CD4+ T-cells (≥80%). During these early time points (12h-day 2), the CD4+ donor T cells were clearly restricted to parafollicular T cell areas where they proliferated, while completely sparing B cell follicles and subepithelial dome regions. On day 4 we observed a rapid emigration of donor T cells from PP, which coincided with enhanced mucosal infiltration of the small bowel, as shown by BLI. These findings correlate well with the picture in mLN, where until day 2 donor CD4+ dominate over CD8+ T cells. Significant changes in the CD4/ CD8 ratio in mLN occurred on day 4 where up to 40 % of the donor T cells were CD8+, while the cellularity remained fairly high.

Experiments with syngeneic luc+ Splc showed preferential homing to the liver, but only transient migration to PP and mLN without signs of proliferation at these sites. After day 4 syngeneic luc+ Splc recipients displayed signs of hematopoietic engraftment.

In summary we showed that homing and proliferation of alloreactive CD4+ donor lymphocytes in specific T cell areas of PP and mLN are crucial events in aGVHD development. FACS analysis revealed distinct changes of the activation markers and homing receptors of the alloreactive T cells. Furthermore BLI provided valuable temporal and spatial guidance for more detailed analysis by IFM and FACS of local environments and enabled us to pinpoint critical events in the induction and extension of aGVHD.

2

THE PARADOXICAL EFFECT OF GLUCOCORTICOID-INDUCED TUMOR NECROSIS FACTOR RECEPTOR FAMILY-RELATED GENE (GITR) ACTIVATION ON ALLOREACTIVE CD4 AND CD8 T CELLS AND THE DEVELOPMENT OF GVHD

Muriglan S.J., Ramirez-Montagut T., Alpdogan O., van Huystee T., Eng J.M., Hubbard V.M., Kochman A.A., Tjoe K.H., van Den Brink, M.R.M. Memorial Sloan-Kettering Cancer Center, New York, NY.

GITR is a member of the tumor necrosis factor receptor family and is involved in regulatory T cell function and TCR activation. GITR is expressed at low levels on resting T cells, B cells and macrophages. Upon activation, CD4+ and CD8+ T cells upregulate GITR expression while CD4+CD25+ regulatory T cells (Treg) constitutively express GITR. Treg stimulation through GITR inhibits their suppressor function and in vivo administration of GITR agonistic antibodies induce autoimmune disease. Here we show that GITR is a relevant molecule for immune responses in graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation (allo BMT). We used a MHC class I/II disparate model for murine allo BMT (C57BL/6 (B6) into BALB/c) to determine the effects of a GITR activating antibody (GITR Ab) on alloreactive T cells during the development of GVHD. We found that addition of GITR Ab to a mixed lymphocyte reaction (MLR) with irradiated BALB/c splenic stimulators, CD4+CD25− B6 effectors and B6 Tregs induced the blockade of Treg suppressor function, as previously described. Interestingly, GITR Ab also had a direct effect on CD4+CD25− and CD8+CD25− T cell populations. Addition of GITR Ab in an MLR showed a 2-fold decrease in CD4+CD25− proliferation while CD8+CD25− proliferation increased by 3.5-fold when compared to the control. Fas deficient CD4+CD25− T cells had an intact alloreactive proliferative response, suggesting that GITR can induce Fas-mediated apoptosis of CD4+CD25− alloreactive T cells. We used the adoptive transfer of CFSE-labeled donor CD3+ T cells into irradiated allogeneic recipients to assess the expression of GITR in vivo and found increased levels of GITR on both CD4+ and CD8+ dividing alloreactive T cells. When we administered the GITR Ab to allogeneic recipients of CD4+CD25− or CD8+CD25− CFSE-labeled T cells, we observed a 45% reduction in the number of donor CD4+CD25− CFSE-labeled T cells recovered at day 3 after infusion. In contrast, there was a 54% increase in the recovery of CFSE-labeled CD8+CD25− T cells. More importantly, mouse recipients of an allo BMT accompanied by CD8+CD25− donor T cells had increased morbidity and mortality in the presence of GITR Ab. Conversely, mice treated with GITR antibody that received allogeneic CD4+CD25− T cells in the allograft showed a significant decrease in GVHD. Our findings identify a novel and paradoxical effect of GITR stimulation on alloreactive T cells during the development of GVHD.

3

EXPANSION OF HUMAN UMBILICAL CORD BLOOD-DERIVED CD34^{+}STEM/ PROGENITOR CELLS TO TREAT MYOCARDIAL INFARCTION

Nagler A.[1], Grynspan F.[2], Peled T.[2], Mandel J.[2], Gutta E.[3], Holbova R.[4], Feinberg M.[4], Leor, J.[4] 1. Hematology, Cord Blood Bank, Chaim Sheba Medical Center, Tel Aviv University, Tel Hashomer, Tel-Aviv, Israel; 2. Gamida-Cell Ltd., Jerusalem, Israel; 3. Danek Gertner Institute of Human Genetics, Chaim Sheba Medical Center, Tel Hashomer, Tel-Aviv, Israel; 4. Neufeld Cardiac Research Institute. Chaim Sheba Medical Center, Tel Aviv University, Tel Hashomer, Tel-Aviv, Israel.

We aimed to test the hypothesis that ex-vivo expanded human umbilical cord blood (CB)-derived Stem/Progenitor cells (S/P) can