P.431

NKT Cells in the Liver are Important for Peripheral Tolerance Induction

Wei Li¹, Zhuonan Li^{1,2}, Zhanpeng Wang¹, Hongyu Liu¹, Lianbo Zhang², Jorge D Reyes³, James D Perkins³

¹Hepatobiliary-pancreatic Surgery, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China; ²Plastic Surgery, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China; ³Surgery, Transplant Services, University of Washington Medical Center, Seattle, United States.

NKT cells are mainly located in the liver in mice. The role of liver inherent NKT cells are still unclear.

Method: In this study, we employed mouse orthotopic liver transplantation and heterotopic heart transplantation models to critical examine the role of liver NKT cells in liver and peripheral tolerance induction.

Results: NKT cells were increased in the tolerated liver grafts which expressed higher levels of CD95L and PD-L1, while the CD4+CD25+Foxp3+ regulatory T cells (Treg) were markedly increased in the both liver and spleen at day 7 post transplantation. The heart allograft survival were prolonged significantly in the recipient which accepted donor spleen cells by portal vein (p.v.) injection in contrast to the recipient which received the donor spleen cells by tail vein (i.v.) injection. In vitro immunological assay revealed that the number of NKT cells in the liver was increased and Treg were increased in both liver and spleen from the p.v. treated mice significantly. The IL-2 expression was decreased, IL-4 and IL-10 were increased. Further, significantly increased IL-4, IL-10, and IFN- γ production was detected from liver NPCs under GalCer stimulation.

Conclusion: NKT cells are important to facilitate transplant tolerance induction, appear to play a key role in down regulation of peripheral immune responses and facilitate CD4+CD25+Foxp3+ Treg induction.

Nature Science Foundation of China (NSFC) (81170416; 81273264). International cooperation fund of Jilin Science & Technology Committee (2016322).

P.432

NK Cells Regulate CD8 T Cell-Mediated Allogeneic Rejection in Immunocompetent Recipients across an MHC Class I Mismatched Barrier

Jose Ignacio Rodriguez-Barboza^{1,6}, Maria Luisa del Rio-Gonzalez^{1,2,6}, Maria Carmen Ferreras³, Leo Buhler⁴, Carlos Fernandez-Renedo^{1,2}, Jose Antonio Perez-Simon^{5,6}

¹Transplantation Immunobiology, University of Leon, Leon, Spain; ²Leon Regional Transplantation Coordination Center, Leon University Hospital, Leon, Spain; ³Department of Animal Health, Mountain Livestock Institute (CSIC) and University of Leon, Leon, Spain; ⁴Visceral and Transplantation Surgery, Department of Surgery, University Hospitals of Geneva and Faculty of Medicine, Geneva, Switzerland; ⁵Department of Hematology, University Hospital Virgen del Rocío/Institute of Biomedicine (IBIS/CSIC), Sevilla, Spain; ⁶Consorcio CIBER-ONC. Grant # CB16/12/00480, Acción Estrategica en Salud 2013-2016, Madrid, Spain.

Non-T natural killer (NK) cells are a population of granular lymphoid cells TcR- CD3- CD122+ (NK1.1+ in C57BL/6 mice and DX5+ in Balb/c mice). These innate lymphoid cells mature in the bone marrow and are responsible for killing viral-infected and transformed cells in a non-MHC restricted manner, without the need of prior sensitization due to a recognition mechanism based on the array of germline-encoded inhibitory and activating receptors expressed on the cell surface of NK cells [1].

NK cells modulate CD8 T cell expansion during homeostatic proliferation in response to lymphopenic conditions by killing activated T cells and competing for IL-15 with the proliferating CD8 effector T cells [2]. This NK cell behavior may respond to an adaptive evolutionary mechanism to prevent aggressive anti-viral immune responses during the acute phase of infection and thus attenuate immunopathology [3].

We set up an experimental mouse model of alloreactivity across an MHC class I barrier to evaluate the role of NK cells in CD8 T cell-mediated rejection of bm1 skin allografts in naïve B6 recipients. In this transplant setting, host CD8 T cells are the main effectors T cells involved in the rejection of class I MHC mismatched skin allografts preferentially through the direct pathway of antigen presentation with limited help from CD4 T cells primed by the indirect pathway of antigen presentation. As opposed to previous studies done in lymphopenic immunodeficient recipients [2], depletion of NK cells was performed in immunocompetent C57BL/6 recipients with or without CD8 T depletion, the former displaying homeostatic proliferation in response to lymphopenia due to antibody-mediated elimination of CD8 T cells. We observed that depletion of NK cells in both CD8-repleted and CD8-depleted naïve C57BL/6 immunocompetent mice accelerated significantly bm1 graft rejection compared to non-NK-depleted controls ($p < 0.0005$) (Figure 1).

Our data claims for precaution in solid organ transplantation under inducing protocols of extensive depletion of leukocytes, such as ATG or alemtuzumab. The immunotherapeutic interventions that include the removal of NK cells may accelerate graft rejection and promote aggressive CD8 T cell cytotoxic responses, which could be refractory to current

FIGURE 1. Host NK cells modulate the early phase of CD8 T cell alloreactive response in immunocompetent recipients. Left panel: Survival curve of bm1 skin grafts in non-depleted and NK-cell depleted B6 recipients. Depletion of NK cells significantly accelerated bm1 skin graft rejection in naïve C57BL/6 mice compared to isotype control (***, $p < 0.0005$). Right panel: Survival curve of bm1 skin grafts into C57BL/6 mice in which depletion of NK cells in CD8 T cell depleted mice accelerated bm1 skin graft rejection compared to CD8 T-cell depletion only (***, $p < 0.0005$).

immunosuppression. It also suggests that enhancing NK-cell functional activity combined with CD8 T cell depletion under tolerogenic regimen that tolerizes CD4 T cells at the time of transplantation may contribute to allogeneic donor-specific tolerance induction by elimination of the direct pathway of antigen presentation and by regulating the recovery of CD8 T cells.

Bibliographic References:

1. Lanier LL. NK cell recognition. *Annu Rev Immunol*. 2005; 23: 225-74.
2. Zecher D, Li Q, Oberbarnscheidt MH, et al. NK cells delay allograft rejection in lymphopenic hosts by downregulating the homeostatic proliferation of CD8+ T cells. *Journal of immunology* (Baltimore, Md: 1950). 2010; 184: 6649-57.
3. Pallmer K, Oxenius A. Recognition and Regulation of T Cells by NK Cells. *Frontiers in immunology*. 2016; 7: 251.

Consorcio CIBER-ONC. Acción Estratégica en Salud 2013-2016, Grant # CB16/12/00480. Acción Estratégica en Salud 2013-2016, Grant # PI16/00002. Consejería de Educación de la Comunidad de Castilla y León. Referencia: Grupos Consolidados de Investigación UIC-012. Referencia: LE003U16. GRS1505/A/17. Gerencia Regional de Salud de la Junta de Castilla y León.

P.433

Standard Induction Immunosuppression Interferes with the Role of Vascularized Bone Marrow in Vascularized Composite Allograft Protection

Nicole Shockcor, Bryan Buckingham, Wessam Hassanein, Arthur J. Nam, Stephen T. Bartlett, Rolf N. Barth
Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, United States.

Introduction: Clinical induction therapies have been demonstrated to interfere with the development of immunologic tolerance in experimental models. Vascularized bone marrow (VBM) has been demonstrated to provide immunologic protection to vascularized composite allografts (VCA). We utilized a non-human primate (NHP) model of facial transplantation to investigate the effects of recipient T cell depletion and steroid therapy on VBM-mediated graft protection.

Materials and Methods: We utilized a well-established model of heterotopic facial subunit VCA in a NHP model. Recipients (N=3) were administered 50 mg/kg of anti-thymocyte globulin (ATGAM) on Day -2, -1, and 0 and transplanted Day 0. Steroids were administered with an initial 20 mg/kg dose and tapered over 21 days and maintained on tacrolimus (Tac) and mycophenylate mofetil (MMF). Chimerism was determined using flow cytometry from peripheral blood samples and final tissues. Comparison was made to historical cohorts, VCA + VBM (n=4) and VCA (no VBM, n=3) treated with Tac/MMF.

Results and Discussion: Mean survival in the ATGAM with steroid taper group was 28.3 ± 8.02 days, as compared to VCA + VBM group 348.2 ± 85.9 days, and VCA (no VBM) 24.7 ± 16.6 days ($p=0.008$). NHP receiving induction therapies developed early Banff 4 rejection, as well as histopathologic evidence of thrombophlebitis and muscle necrosis. In contrast to the VCA + VBM group, chimerism was undetected in peripheral blood and graft bone marrow was nearly acellular, with an average of $91.5\% \pm 2.4$ (range 89.8-93.2%) of the lymphocytes present recipient in origin (n=2). Also in contrast to VCA + VBM but comparable to VCA (no VBM), the development of IgM and IgG donor specific antibodies was noted in the ATGAM with steroid taper group, with a mean of 357.5 and 180.5 MFI respectively.

FIGURE 1.

Conclusion: Presumptive VBM cell populations that regulate immune responses are susceptible to T cell depleting and steroid therapies. The observed interference of standard induction therapies on the protective value of VBM highlights relationships between pharmacologic and biologic mechanisms of VCA graft protection.

Henry Jackson Foundation, Department of Defense Award # W81XWH-13-2-0056. Astellas Pharma US, Inc.