Modulating Endogenous CD4+ T cell Restoration Following Sepsis

Jeff Babcock1, Javier Cabrera-Perez1,2, Britnie James3, Tamara Kucaba1, Erik Brincks, and Thomas S. Griffith1,2,3,4
1. University of Minnesota Medical School, 2. Microbiology, Immunology & Cancer Biology Graduate Program, 3. Department of Urology, University of Minnesota Medical School, Minneapolis, MN, 4. Veteran’s Affairs Hospital, Minneapolis, MN

Background
The phenomenon of septicemia often leaves surviving patients with detrimental immunosuppressive sequelae resulting in nosocomial burden. These secondary infections following the acute phase of sepsis kill more than 200,000 hospitalized patients annually1. Although survivors restore total lymphocyte cell counts after several weeks, the efficacy of the adaptive immune system is impaired. A significant influence on this impairment can be contributed to the changing profile of the CD4+ T cell population in a given system. While definitive causative agents have not been identified, reactive oxygen species and metabolic stress are candidates when considering contributors to this lymphopenic state. The surviving CD4+ populations undergo homeostatic proliferation to restore bulk numbers, however, the diversity in the repertoire of TCRs can decrease dramatically. Additionally, remaining cells can enter an anergic-like state where effector functions and phenotype plasticity can be limited. With newly acquired deficits in CD4+ responses, patients are left susceptible to a variety of bacterial and viral infections2. Many interleukins are critical for T cell development and proliferation. IL-2 has already been implemented in treating patients with severely compromised T cell function. These cytokines may also have the potential to assist T cells rebounding from a septic environment.

Hypotheses
1) Cytokine therapy (e.g. IL-2 or IL-7) after sepsis augments CD4+ T cell recovery and function.
2) CD4+ T cells reactive to intestinal flora expand following sepsis.

Methods
A) Caecal ligation and puncture (CLP)
B) CD4+ T cells
C) CD44hi IL-7Rαhi T cells

Cytokine Therapy
1. CD4+ T cells
2. CD44hi IL-7Rαhi T cells

Summary of Findings
1) IL-2 and IL-7 complex treatments enhance numerical recovery of bulk CD4+ T cells.
2) Enhancement of antigen-specific CD4+ splenocyte recovery from sepsis using interleukin therapy is cytokine-selective.
3) Variability in gut microbiota influences the recovering capacity of antigen-specific T cell populations after sepsis.

Acknowledgements and References
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OVERCOMING IMMUNE CELL ASSOCIATED TUMOR VASCULATURE DISRUPTION THROUGH NORMALIZING THE TUMOR VASCULATURE IN B16 MELANOMA TUMORS

Brendan Coutu, Brandon Burbach, Matt Mescher, Yoji Shimizu
University of Minnesota, Center for Immunology

Introductory Work

- Through preliminary work within the Shimizu lab, an association was suspected between CTLs and a disruptive effect on tumor vasculature.
- In this introductory work we sought to show that there is a tumor vasculature disruption associated with adoptively transferred effector CTLs specific for a tumor antigen.
- Using both Doppler ultrasound and PECAM-1 histology staining we were able to show this association in a B16-OVA tumor model treated with in-vitro activated OT-1 effector cells.

Overcoming Tumor Vasculature Disruption

Abstract

The success of Adoptive Cell Transfer (ACT) is dependent on the localization of tumor specific cytotoxic lymphocytes (CTL) to the tumor. Tumor specific CTLs show only moderate tumor invasive capabilities as the CTLs access to a tumor diminishes after an initial phase of adoptive immunotherapy due to an associated disruption in tumor vascular. We set out to show that through normalizing the tumor vasculature by priming the tumor with Axitinib, an angiogenesis inhibitor, the tumor vasculature is increasingly resistant to ACT associated vasculature disruption. Increased tumor control and tumor localization of both transferred and host immune cells is achieved through the synergistic effect of Axitinib and ACT.

Introduction

- To sustain rapid growth, tumors promote angiogenesis resulting in the formation of a highly disordered capillary system characterized by dilated, leaky, and tortuous blood vessels.
- It has been noted that at a moderate dose an angiogenesis inhibitor can normalize the tumor vasculature into a highly ordered capillary system with increased tissue perfusion.
- In order to promote tumor vascular normalization we used Axitinib, a small molecule inhibitor of the VEGF receptor which is involved in angiogenesis promotion in melanoma tumors.

Experiment Set-Up

Figure 1: Tumor Vasculature disruption is associated with CD8 effector CTLs.

Results

Figure 2: Axitinib is a Small Molecule Angiogenesis Inhibitor.

Figure 3: Tumor size is synergistically controlled by Axitinib and OT-1 effector cells.

Figure 4: Phenotypic differences can be visualized in excised tumors by day five post-treatment.

Figure 5: Doppler ultrasound can detect vascular changes associated with each treatment.

Conclusion

- Tumor vasculature disruption associated with ACT can be overcome through vascular normalization using Axitinib, an angiogenesis inhibitor.
- Sustained tumor control can be achieved through the synergistic effects of ACT and Axitinib.

Future Directions

- Utilize histology slides stained with PECAM-1 to investigate the vascular changes at the capillary level.
- Assay the amenability of OT-1 localization to the Axitinib treated tumor by retransferring splenic OT-1 cells on day seven post original OT-1 treatment.
- Assay the ability of splenic OT-1 cells to localize to an Axitinib treated tumor by transplanting tumors between two mice with different OT-1-congenic markers.

References


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Influence of Cytokines in NK Cell Proliferation After Double Umbilical Cord Blood Transplantation

Alyssa Kerber, Rachel J. Bergerson, Michael R. Verneris
Division of Hematology, Oncology and Blood and Marrow Transplantation in the Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota, USA

Background

• Double umbilical cord blood (dUCB) transplantations are used to treat patients with hematopoietic malignancies.
• NK cells are the earliest lymphocyte population to recover after dUCB transplant.
• Our preliminary results show that high NK cell proliferation at day 28 leads to reduced relapse and increased disease-free survival.
• The cytokines IL-2, IL-15, and IL-7 support the generation of NK cells in vitro.
• IL-15 is typically regarded as the most central and critical cytokine supporting NK cell development.
• IL-15 and IL-15Ra mice lack NK cells.

Hypothesis

Patients with high NK cell proliferation at day 28 post-dUCB transplant will have elevated serum levels of cytokines IL-2, IL-7 and IL-15 at day 7 and/or day 28 post-transplant.

Methods

• Study population was 53 patients treated at the University of Minnesota between 2010-2012 undergoing dUCB.
• Clinical parameters were collected and housed in the University of Minnesota Blood and Marrow Transplant Database.
• Clinical samples were analyzed for NK cell proliferation using Ki67 staining and other lineage markers (CD3, CD56, etc).
• Patient serum samples from days 7 and 28 post-dUCB transplant were analyzed for levels of IL-2 and IL-15 using eBioscience Ready-Set-Go ELISA kit and IL-7 using R&D Dusset ELISA kit.
• Results were analyzed with unpaired t-test and Spearman’s rank correlation coefficient.

Conclusion & Future Directions

• There was no significant difference in the levels of IL-2 or IL-7 between high vs. low NK cell proliferators.
• IL-15 levels at day 7 are significantly elevated in patients with high NK cell proliferation at day 28 post dUCB transplant.
• IL-15 levels at day 28 are significantly lower in patients with high NK cell proliferation at day 28 post dUCB transplant.
• Future directions include comparing function and maturation of NK cells based on levels of IL-15.
• We will also assess NK cell response by measuring levels of STAT5 phosphorylation following stimulation of patient cells with IL-15.

This novel data supports administering IL-15 to patients immediately after transplant to trigger an increase in NK cell proliferation, and presumably, better clinical outcomes.
Effects of Wnt pathway agonist TWS119 on CD8+ T lymphocyte activation and phenotype

**Abstract**

The achievement of complete remissions in patients with acute lymphoblastic leukemia (ALL) following adoptive cell transfer (ACT) of chimeric antigen receptor-modified T cells (CAR-T) has elevated ACT as a promising platform for the treatment of many forms of cancer. Subsequent research in both humans and animal models has established a strong correlation between various outcome measures — including engraftment potential, tumor regression, and overall survival — and the increased abundance of less differentiated CD8+ T lymphocyte subsets among the transferred T cell population. This relationship has spurred interest in developing the ability to generate activated, CAR-modified CD8+ T cells in vitro that are enriched in CD44hiCD62LloSca-1hiB220hiCD122+ stem cell memory (TSCM) and CD44hiCD62LloSca-1hiCD122+ effector memory (TEM) subpopulations. Production of TSCM and TEM via Wnt pathway agonism, either through receptor-mediated activation or downstream potentiation via glycogen synthase kinase 3b (GSK-3b) inhibition, has been reported. Here we characterize the effects of GSK-3b inhibitor TWS119 on CD8+ T cell proliferative potential and phenotype. CD8+ T cells were isolated from spleen and lymph nodes of naive TCRαβ+ T cells harvested from WT C57BL/6 mice and activated using anti-CD3ε/CD28-conjugated beads in the presence of IL-2. Consistent with previous reports, TWS119 blocked the generation of the CD44hiCD62LloSca-1hiCD122+ effector memory (TEM) subpopulation, in favor of CD44hiCD62LloSca-1hiCD122+ phenotypes at concentrations as low as 1 μM. Higher concentrations of TWS119 further promoted the CD44hiCD62LloSca-1hiCD122+ phenotype, however a subset of this group did not reach high levels of Sca-1 expression, suggesting only a portion of these cells belong to the TSCM population. Additionally, TWS119 treatment induced a dose-dependent reduction in T cell proliferative capacity. Future work will further optimize the use of TWS119 in vitro expansion system as well as combing low dose TWS119 with complimentary approaches to TWS119 generation, including substitution of IL-2 with IL-15 and IL-15+IL-2 during activation.

**Background and Significance**

T lymphocytes develop from CD34+CD38− naïve (T0) cells towards more differentiated CD44hiCD62LloSca-1hiB220hiCD122+ effector memory (TEM) subpopulations. A less differentiated stem cell-like memory subset (TSCM) has more recently been recognized. In mice, TSCM cell retain a TCRαβ phenotype (CD4+CD62L), but are antigen-experienced and distinguishable from T0 cells due to elevated expression of CD122 (IL-2 receptor β chain), stem cell antigen-1 (Sca-1), and the anti-apoptotic protein Bcl-2. During development, T0 cells are the focus of intense research interest due to their enhanced engraftment potential, increased capacity for self-renewal and unique ability to generate both T0 and TCM populations as well as fully differentiated effector cells (TEMs). Furthermore, TCM populations confer greater antitumor activity and facilitate increased duration of remissions when used for adoptive T cell transfer therapy.

**Isolation and Purification**

CD4+ and CD8+ T lymphocytes were isolated and purified from spleen and lymph nodes harvested from WT C57BL/6 mice. Purified T0 cells were activated with anti-CD3ε/CD28-conjugated beads (Life Technologies) and expanded in complete RPMI supplemented with 10 ng/mL recombinant human IL-2 and either:
- Vehicle control (DMSO)
- 1, 3 or 5 μM GSK-3b inhibitor TWS119

**Experimental Conditions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD4+ Subpopulation Phenotype</th>
<th>In Vitro Proliferative Capacity</th>
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<tbody>
<tr>
<td>Vehicle Control</td>
<td>CD44hiCD62LloSca-1hiB220hiCD122+</td>
<td>Day 6: 1.5x Day 0</td>
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<tr>
<td>1 μM TWS119</td>
<td>CD44hiCD62LloSca-1hiB220hiCD122+</td>
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<tr>
<td>5 μM TWS119</td>
<td>CD44hiCD62LloSca-1hiB220hiCD122+</td>
<td>Day 6: 0.9x Day 0</td>
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**Conclusions**

Low dose TWS119 efficiently blocks generation of CD8+ TCM populations in favor of TSCM and TEM populations. TWS119 treatment results in a dose-dependent decrease in T lymphocyte proliferative capacity. Increasing concentrations of TWS119 correlate with decreased expression of Sca-1 in the CD44hiCD62LloSca-1hi subset, suggesting the anti-proliferative activity of TWS119 may limit the expansion of the TSCM subset.

**Future Directions**

Further optimize the TWS119 procedure for TCM generation using the bead-based activation/expansion system. Combine low dose TWS119 treatment with complimentary approaches toward T0 production, including IL-15 substitution for IL-2. Assess CAR transduction efficiency in TWS119-treated CD8+ T cells.

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**References**

The role of Th17 cells in cancer development remains enigmatic, with diverse cancer models yielding conflicting results supporting both the inhibition and promotion of tumor progression. The effects of IL-17 are pleiotropic and dependent on tumor milieu, but have not been explored in B Acute Lymphoblastic Leukemia (B-ALL). We recently established a population of BCR-ABL specific CD4+ T cells which respond to BCR-ABL B-ALL. To examine the role of IL-17 in BCR-ABL B-ALL we injected this leukemic strain or BCR-ABL peptide (BAP) with Complete Freund’s Adjuvant (CFA) into IL-17GFP reporter mice. We used BAP-I-A^b-specific mHIC1 tetramers to identify and characterize the CD4+ T-cell cytotoxic response under these conditions. We observed a trend towards increased IL-17 production in BAP-I-A^b-specific T cells in response to BAP CFA injection and in leukemia immunized mice compared to the bulk population of CD4+ T cells. IL-17 production by BAP-I-A^b-specific T cells was negatively correlated to leukemia burden but was increased in mice immunized with a higher load of leukemia cells compared to a smaller load. BAP-I-A^b-specific T cells that expressed a chronically-stimulated phenotype secreted less IL-17 than BAP-I-A^b-specific T cells expressing an acutely-stimulated phenotype. In conclusion, we discovered that some leukemia-specific T cells polarize into the Th17 lineage in response to leukemia, that IL-17 production by leukemia-specific T cells decreases in correlation with leukemia progression, and finally that chronically-stimulated leukemia-specific T cells have decreased capacity to produce IL-17.