Effects of limiting citrate derived Acetyl-CoA production on the development of exhaustion by CD8+ T cells

Presentation by Alessandra Azure

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Healthy T cells kill cancer cells. They promote cancer patient survival.

- T cells are part of the adaptive immune system – they are specialized
  - **CD8⁺ T cells** are “killer” T cells that kill virally infected and cancerous cells
  - T cell produce **cytokines**, which are signals cells use to communicate
    - IFNγ and TNF-α are pro-inflammatory

- CD8⁺ T cells can become **exhausted**
  - Exhaustion = hypofunctional state where some anti-tumor effector function is lost
  - Associated markers: PD-1, TIM-3

**What causes T cell exhaustion?**

Ma, J. et al. Immunotherapy Cancer, 2019
Chronic activation and the suppressive TME drive T cells to exhaustion.

Conditions of the tumor microenvironment (TME):

- Nutrient insufficiency
- Low oxygen (hypoxia)
- Waste buildup
- Increased presence of inhibitor receptor ligands
- Immunosuppressive Treg cells

Persistent antigen exposure and the harsh TME cause T cells to become **exhausted**

DePeaux, K., Delgoffe, G.M. Nat Rev Immunol, 2021
Exhausted T cells change the way they produce energy.

- Exhausted T cells lose full **mitochondria** function
- Causes **metabolic insufficiency**
- Cells shift metabolism to alternate pathways outside mitochondria – possibly **fatty acid synthesis pathway**

**Lipid droplet**

- CD8+ LN
- CD8+ TIL PD1mid
- CD8+ TIL PD1+Tim3+

Less exhausted → More exhausted

- **Fatty Acids** are one type of nutrient that provides energy to T cells; **lipid droplets** store fatty acids
- Dysregulation of the fatty acid synthesis pathway may be detrimental to T cells

How is lipid accumulation related to the progression of T cells towards exhaustion?

Nicole Scharping
Cheon, S. et al. Journal of Molecular Medicine, 2021
Does inhibiting citrate derived Acetyl-CoA production impact the development of exhaustion in CD8^+ T cells?
Methods: Evaluating ACLYi in vitro

**Experiment Goal:** Investigate the impact of inhibiting Acetyl-CoA production on the development of exhaustion by CD8+ T cells.

- **T cell receptor (TCR)** was activated with αCD3/αCD28 dynabeads
- 6 day CS period
- **Media contains ACLYi at appropriate experimental concentration** (dosage not yet established)
- Analyzed via flow cytometry – single cell analysis of surface markers and cytokines
ACLyi and chronic stimulation decrease cell proliferation

As inhibitor concentration increased, cell proliferation decreased, possibly due to **metabolic stress**.

Chronically stimulated cells proliferated less than acutely stimulated cells.
No notable change in PD-1 or TIM-3 expression was observed at any inhibitor concentration.

DP = PD1$^{hi}$ TIM3$^{hi}$

TIM-3 and PD-1 are surface markers associated with T cell exhaustion.
Cytokine production indicates the functional capacity of T cells.

- IFNγ and TNF-α are pro-inflammatory cytokines that stimulate an immune response.

DN = double negative
DP = double positive

Unstimulated controls

Method: Cytokine Intracellular Staining

1. Add cytokine-specific antibodies + Analyze by flow cytometry
2. Fix and permeabilize
3. Activate T cells + Add protein export inhibitor
Observed **increase** in cytokine production in cell groups treated with 40 μM ACLYi.

Restim TCR with αCD3/αCD28
DP = IFN$^\text{hi}$ TNF$^\text{hi}$

<table>
<thead>
<tr>
<th>Concentration</th>
<th>AS</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0μM</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (47.3% DP)</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (5.39% DP)</td>
</tr>
<tr>
<td>10μM</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (50.1% DP)</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (11.3% DP)</td>
</tr>
<tr>
<td>20μM</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (50.0% DP)</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (11.0% DP)</td>
</tr>
<tr>
<td>40μM</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (61.7% DP)</td>
<td>![IFN$^\text{hi}$ TNF$^\text{hi}$] (51.0% DP)</td>
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Putting it together

• Limiting Acetyl-CoA production may impact CD8+ T cell effector function
  • Sustained cytokine signaling would promote antigen presentation and recruitment of immune cells

• Optimal ACLYi concentration likely lies around 30µM
  • Higher inhibitor concentration = less cell proliferation, more cytokine impact

• May be possible that ACLYi is delaying T cell progression to complete/terminally differentiated exhaustion

Zhang, J. et al. FASEB, 2021
Future Direction

- Hypoxia repeat testing (1.5% O₂); Microscopy
- In vivo mice tumors
  - Investigate how surface marker expression interacts with cytokine production
  - Combination with anti-PD1 immunotherapy

- **Big Picture:** Make cancer patients’ T cells work better for longer
  - Better way of treating cancer
  - Early clinical trial ongoing with similar inhibitor

Metabolism!

*Crown Bioscience, 2021*
Acknowledgements

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**Hillman Academy**
- Dr. David Boone
- Steven Jones
References


DePeaux, K., Delgoffe, G.M. Metabolic barriers to cancer immunotherapy. Nat Rev Immunol 21
References cont.


“Study of MTB-9655, an Inhibitor of ACSS2, in Patients With Advanced Solid Tumors.” ClinicalTrials.gov. ID: NCT04990739


Appendix – All Cytokine unstim controls

Unstimulated controls

TNF-α

IFNγ
Appendix - Gating

Unstim AS 0uM

AS 0uM

CS 0uM

CS 40uM

Gating (1) lymphocytes
(2/3) singlets
(4) live cells
(5) CD8⁺ T cells

AS = acute stimulation
CS = chronic stimulation
Appendix - Cytokines

CS; aCD3/aCD28 restim

Observed noticeable increase in cytokine production in cell group treated with 40 μM ACLYi concentration.

IFNγ and TNF-α are pro-inflammatory cytokines that stimulate an immune response.
Appendix – Flow Cytometry

- Cells were analyzed for surface markers, intracellular markers, and cytokine production via **flow cytometry**

A flow cytometer performs single-cell analysis of stained markers.
The Effects of Limiting Citrate-Derived Acetyl-CoA Synthesis on the Development of Exhaustion in CD8\(^+\) T Cells

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**INTRODUCTION**

- CD8\(^+\) T cells are a subset of T cells that kill cancer cells
- IFN\(\gamma\) and TNF-\(\alpha\) are pro-inflammatory cytokines. They promote an anti-tumor immune response

**METHODS**

- CD8\(^+\) T cells were isolated via negative selection using MojoSort™ Streptavidin Nanobeads
- T cell receptor (TCR) was activated in vitro with CD3/CD28 Dynabeads™ (+ muL-2)
- Acute Stimulation (AS) and Chronic Stimulation (CS) groups
  - AS = 24-hour TCR stimulation
  - CS = 6 days continuous TCR stimulation
- Media contains ATP Citrate Lyase inhibitor (ACLY)
  - BMS-303141
  - 4 concentrations used; 4\(\mu\)M = control
  - All cells were analyzed using flow cytometry
  - Cytokine staining included un-restimulated controls; a protein export inhibitor was added to cells prior to restimulation and cytokine staining
  - N = 2 full repeats

**RESULTS**

- **Cell Growth**
  - As inhibitor concentration increased, cell proliferation decreased, possibly due to metabolic stress. As expected, chronically stimulated cells proliferated less than acutely stimulated cells.

- **Cytokine Production of IFN\(\gamma\) and TNF-\(\alpha\)**
  - Cells treated with 40\(\mu\)M of ACLYi consistently produced notably more pro-inflammatory cytokines (IFN\(\gamma\) and TNF-\(\alpha\)) than cells treated with lower inhibitor concentrations or no inhibitor.

**CONCLUSIONS**

- Inhibiting Acetyl-CoA synthesis does impact CD8\(^+\) T cell effector function
- Sustained cytokine signaling would promote antigen presentation and recruitment of immune cells – therefore improving the body’s immune response against cancer
- Little to no observed impact on phenotypic exhaustion
- Optimal ACLYi concentration likely lies around 30\(\mu\)M
- Higher inhibitor concentration = less cell proliferation; more cytokine impact
- May be possible that ACLYi is delaying T cell progression to terminally differentiated exhaustion
- Results provide evidence to the merit of investigating lipid accumulation as a driver of exhaustion

**FUTURE DIRECTIONS**

- Repeat testing under hypoxia (1.5% \(O_2\)) to mimic conditions of the tumor microenvironment
- Use microscopy to visualize changes in lipid droplet size
- Making the jump to vivo:
  - Test CD8\(^+\) T cells treated with ACLYi in in vivo mouse tumors; observe rates of IFN\(\gamma\) and TNF-\(\alpha\) production in TILs
  - Pair with a PD-1/PDL-1 blockade therapy; mitigate immunosuppressive effects of PD-1 binding
- Bench to bedside transition:
  - Current First-In-Human clinical trial use a similar, orally-available inhibitor of Acetyl-CoA production (via ACSS2) in patients with advanced solid tumors
  - Immunotherapy potential

**REFERENCES**


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