Investigating Myc-Knockout T Cells to Re-engineer Immune Response

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Abstract

Upon chronic or long-term stimulation of antigen proteins during infections, T cells (CD8+) are limited in their function as they differentiate into exhaustive states. During activation, T cells reprogram their metabolic pathway to use glycolytic, pentose-phosphate, and glutaminolytic pathways. Myc transcription factor is induced upon T cell activation and highly involved in metabolic pathway linking glutaminolysis to biosynthesize energy for T cells. There is growing evidence that shows Myc plays an essential role in development, differentiation, and activation of immune T cells as it progresses between progenitor-like and exhaustive states. This study investigates the effect of Myc-deleted gene in T cells as it undergoes a chronic antigen response simulated by *in vitro* T cell exhaustion assay. Flow cytometry analysis revealed that more Myc-knockout (Myc-KO) T cells expressed Tim3, which is associated with terminal exhaustion. Furthermore, more control cells expressed CD62L, indicating that Myc is involved in T cell progression towards effector-like state to memory T cells. By understanding the role of Myc in T cell activation, we can manipulate the immune response to avoid overstimulation and exhaustive states and improve immunotherapies for chronic disease.

Background

Focus: T-cell metabolism during activation upon chronic infection can lead to programmed cell death and terminal exhaustion. Observe Myc's critical role in metabolic reprogramming in T cells.

- T-cell Exhaustion: rapid proliferation and expansion requires bioenergy.
- Activation of T cells rapidly switches metabolic programs from fatty acid β-oxidation and pyruvate oxidation via the TCA cycle to aerobic glycolysis, PPP and glutaminolysis.
- Myelocytomatosis oncogene (Myc)- transcription factor that promote expression of target genes that coordinate death, proliferation, and cellular metabolism
  - Involved in the development and differentiation of immune T cells
  - Activation of T cells triggers a Myc-dependent induction of glutaminolysis → upregulates transporters that carry glutamine into cells to use for energy
  - Levels of Myc increase progenitor differentiation post-transcriptionally
  - Myc inhibits terminal differentiation of most cell types and sensitizes cells to apoptosis
- Myc-deficient T cells have defects in glucose and glutamine metabolism

Goal: By deleting the Myc gene in immune cells, this experiment aims to understand the role of Myc in the progression of CD8+ T-Cell exhaustion between progenitor-like and terminal exhaustion states.

Role of Myc Trans Factor in CD8+ T cells

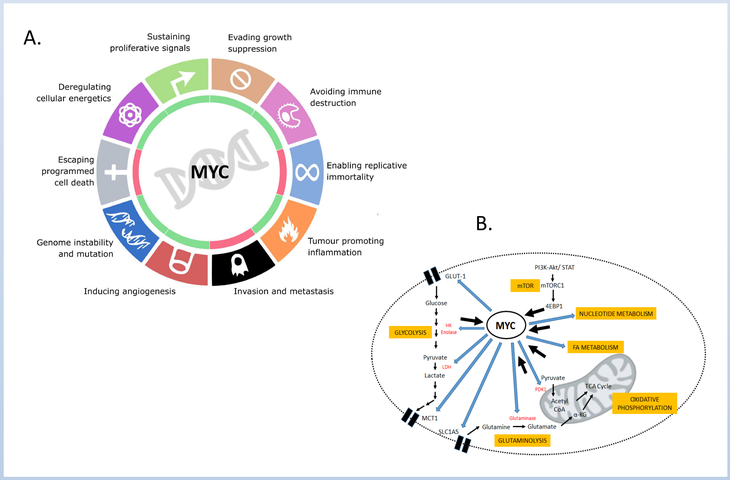

Figure 1. Role of Myc in metabolic function of activated T cells. A) Myc was first identified as a family member in the human genome that was connected to cancer growth as an oncogene. Since then, extensive research has revealed its diverse role in regulating innate and adaptive immunity as well. It is involved with immune cell maturation, activation, proliferation, and polarization. (Llombart, et al., *The Lancet,* 2021) B) Myc is a transcription factor that controls metabolic functions of glutaminolysis to synthesize polyamines in activated T cells. Glutamine supplies metabolic intermediates through carbons and nitrogen to promote cell growth/proliferation. It also prevents oxidative stress during proliferation. Once a T cell is activated, downstream signaling induces higher levels of Myc protein production, which is needed proliferation, survival, and differentiation of immature thymocytes. Early expression of Myc promotes inflammatory cytokines to induce positive feedback loop to sustain immune response to pathogens. However, inactivation of Myc in T cells can result in apoptosis and suppressing differentiation and maturation. (Sabnis, et al., *Genes*, 2017).

Methods

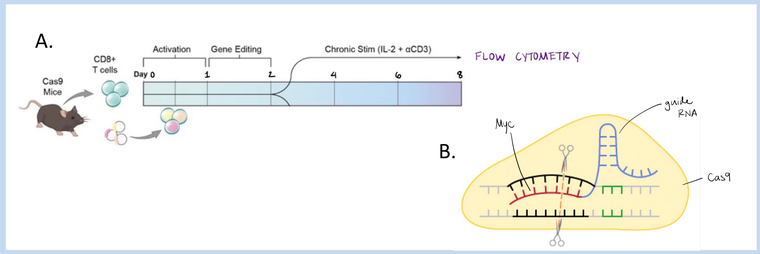

Figure 2. T cell exhaustion assay and Myc deletion. A) Spleen cells collected from C57BL/6J mice with Cas9 gene and CD8+ T cells isolated and enriched. Cells were seeded at a concentration of 1 million cells/mL on plates coated with 1 μg/mL anti-CD3 and 1 μg/mL of anti-CD28. Cells were kept on activation plates for 48 hours prior to the experiment. To induce T cell exhaustion, chronic stimulation was performed using 5 μg/mL CD3 and 10 ng/mL IL-2. Cell were passaged onto a fresh coated plate every 2 days. B) CD8+ T cells were transduced with guide RNA through concentrated retrovirus 24 hours after isolation to delete the Myc gene. Myc is conditionally deleted using Tamoxifen (4OHT), which binds to estrogen receptor to turn on CRE production and then the Cas9 production. Guide RNA binds to the Cas9 and is instructed to cut out the Myc gene.

T Cell Exhaustion

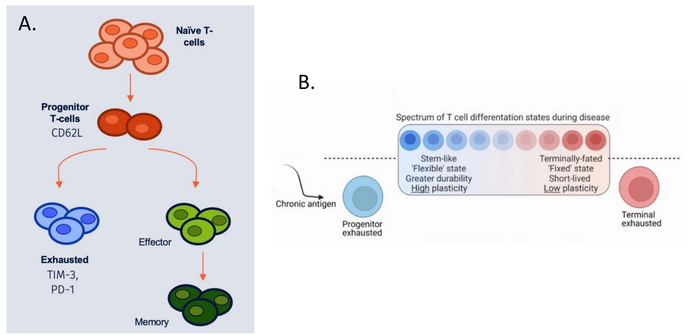

Figure 3. T cell differentiation during chronic antigen response. A) T cell exhaustion results from chronic stimulation of T cell receptors during immune response, which causes low cell proliferation and poor response to tumor antigens. T cell activation causes up-regulation of uptake of glutamine and glucose (glycolysis) after stimulation of CD3 and CD28 receptors in thymocytes and mature lymphocytes. This activation leads to metabolic reprogramming of a T cell to use glycolysis, PPP, and glutamine oxidation, which involves global regulation of metabolic gene transcription. B) Studies have shown that amount of Myc expression is directly proportional to the glycolysis that occurs during T cell activation in order to increase biosynthesis for energy consumption. T cells undergo a spectrum of differentiation that could result in stem-like progenitor cells with higher plasticity, or terminal exhausted cells that are short-lived. Recent studies have shown that T cell exhaustion has largely become a barrier in improving the efficacy in immunotherapies such as CAR-T cell immunotherapy. Understanding and manipulating this process can allow for improved functionality of T cells in immune response and higher efficacy in cancer.

Myc-KO vs. Control Terminal Exhaustion (Tim3)

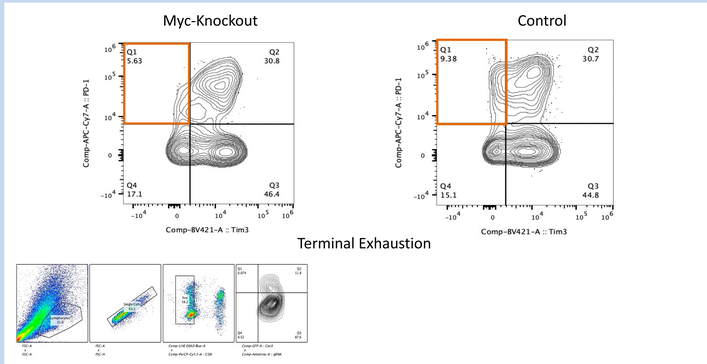

Figure 4. Myc-knockout CD8+ T cells have more terminal exhaustion than control cells. PD1 and Tim3 are commonly seen in exhausted T cells, where Tim3 indicates more terminal exhaustion and PD1 is initial onset. Their co-expression has been associated with more exhausted phenotypes of T cells and typically increases over time during chronic infection. Expression of PD1 and Tim3 is linked with impaired proliferation, low cytokine production, and low cell survival, which result in diminished ability for CD8+ T cells to control viral attack. The level of exhaustion seems to skew towards Tim3 or terminal exhaustion in CD8 T cells with Myc-knockout, while there is less terminal exhaustion in control cells. This aligns with the integral role of Myc in regulation metabolic pathways that sustain T cell proliferation and function during antigen response. With low/no Myc levels, there is minimal transportation of necessary amino acid for biosynthesis and energy production, which inhibits T cell function.

Myc-KO vs. Control Progenitor Exhaustion (CD62L)

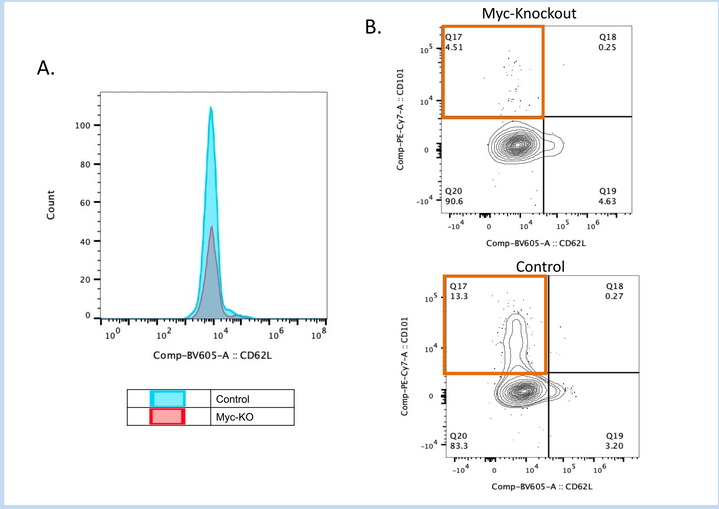

Figure 5. Level of CD62L expression indicates less progenitor exhaustion in Myc-knockout CD8+ T cells. A) T cells that express CD62L retain the high proliferative potential and can eventually lead to differentiation into effector and memory immune cells. CD62L+ T cells maintain their potential for expansion and typically have low PD-1 expression levels. Myc-KO T cells expressed lower CD62L compared to the control, suggesting that Myc plays a role in effector cell differentiation. Without Myc, T cells were likely to undergo apoptosis or programmed cell death. B) The overactivation of T cells during exhaustion can lead to expression of CD101, which can result in an irreversible exhaustive state of CD8+ T cells. Interestingly, Myc-KO expressed slightly higher levels of CD62L compared to control cells. This could be due, however, to the small sample size (n=2) and disproportionate number of Cas9 deleted Myc cells. It seems that in a typical T cell, exhaustion levels reach overactivation with higher levels of CD101 expression. Myc-KO cells could be undergoing more apoptosis before it even reaches this state of exhaustion.

Conclusion and Future Research

- Myc-knockout T cells resulted in higher expression of terminal exhaustion noted by TIM3 expression.
- Control T cells expressed more CD101 and CD62L, indicating Myc's involvement with exhaustive state of T cell differentiation and programmed apoptosis.
- CD8+ T cell exhaustion assay relatively effective in observing gene knockout.
- Potential change to experiment: turn on as much Cas9 as possible to effectively delete Myc gene by adding 4OHT earlier.
- Understand Myc functions in T cell immune response to potentially reverse T cell exhaustion.
- Target Myc or downstream metabolic programs to advance treatment of immunological disease.
- Improve efficacy of immunotherapies.