Immunology & Cell Biology 2022; **100**: 83–86
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NEWS AND COMMENTARY **OPEN**

Mastering an exhausting marathon: how CD8$^+$ T cells fine-tune metabolic fitness

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Immunology & Cell Biology 2022; **100**: 83–86; doi: 10.1111/imcb.12517

Long-term antigen exposure in chronic infection and malignant tumors induces CD8$^+$ T cells with an exhausted or dysfunctional phenotype.[1] Such T cells characteristically show high expression of PD-1 and other inhibitory factors, impaired production of effector cytokines such as IFNγ and TNFα, and reduced effector capacity. This attenuated effector state prevents the onset of severe immunopathology in persisting infections while it allows T cells to still mediate a residual, but critical, level of pathogen control.

In recent years, the field has made significant progress in understanding the dynamics and function of T cells in chronic infection and has identified key transcriptional mechanisms that initiate and maintain exhausted T cell populations. These include transcription factors such as Tox, Irf4, Tcf-1, Nfatc1, Batf, Bach2, Tbet, Eomes and Nr4a.[2–10] Moreover, we know now that the population of exhausted T cells is very heterogenous and consists of Tcf-1 expressing progenitors of exhausted T cells (Tpex) and terminally differentiated effector cells (Tex) that are Tcf-1$^-$. These progenitors are critical for maintaining the anti-viral immune response, as they continuously generate Tcf-1$^-$, exhausted effector T cells. Moreover, Tcf1$^+$ progenitors appear to be the key cells that respond to checkpoint inhibition[11] by generating tumor-targeting Tcf-1$^-$ effector cells. An important question that has remained underexplored was which specific metabolic pathways support the function and differentiation of Tpex and their descendants.

For naïve T cells, it is known that their metabolism shifts in acute infection from a dominance of catabolic oxidative metabolism to an anabolic metabolism that involves high glycolytic activity. Compared with effector T cells, exhausted T cells appear metabolically impaired, or at the very least significantly altered, during chronic infection, with these T cells displaying an overall reduced mitochondrial and glycolytic metabolism.[12] Of note, exhausted T cells differ substantially from the T cells found in acute infection as they persist in a continuously activated state over long periods of time, while T cells in acute infection rapidly return from a highly activated state to a resting state. The extent to which the altered metabolism seen in exhausted T cells influences the specific response pattern of exhausted T cells has remained unclear in critical parts, although clear evidence was recently provided that re-activation of exhausted T cells goes along with metabolic re-programming.[13] Moreover, it is well known that precursors irreversibly transition from a stem-like phenotype into cells that have a short or limited lifespan (Figure 1), but to date which metabolic pathways are linked to this transition has remained largely unknown. Here, the paper published by Gabriel *et al.*[14] provides exciting new insight as the authors report a metabolic master "switch" that regulates the differentiation of T cells during chronic viral infections.

In their publication, the authors provide a detailed description of the metabolic particularities of exhausted T cells during chronic lymphocytic choriomeningitis virus (LCMV) infection. Using ID3-GFP mice, which allows the faithful identification of Tpex (Id3$^+$Tim-3$^-$) and Tex (Id3$^-$Tim-3$^+$) cells, they showed that in comparison with Tex, Tpex displayed enhanced metabolic fitness. This was characterized by a high mitochondrial mass, enhanced mitochondrial membrane potential and a high spare respiratory capacity – the ability of cells to respond to increased energy demands by increasing respiration. Interestingly, mitochondrial spare respiratory capacity has been associated with long-term proliferative potential and cellular plasticity in the context of recurring infections, where a high spare respiratory capacity marks memory cells with recall potential.[15] In line with this concept, Id3$^+$ precursor cells with long-term proliferative potential were able to

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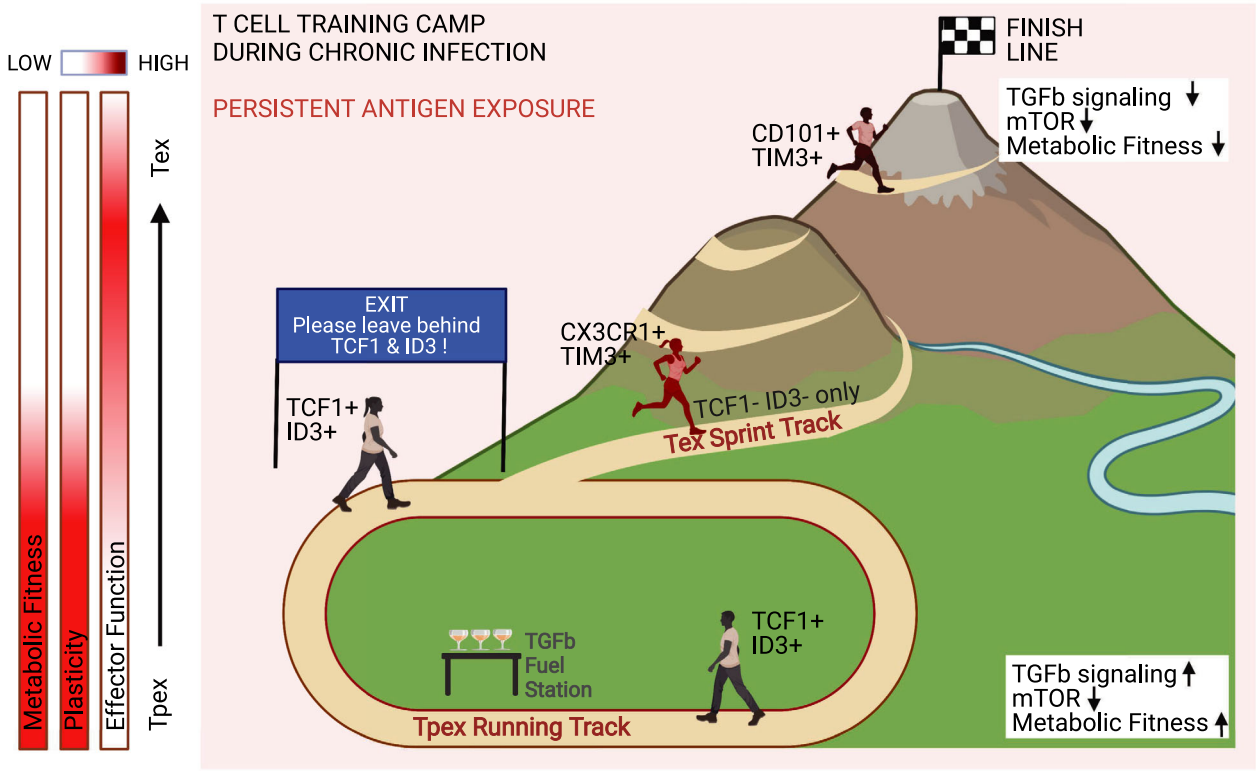

Figure 1. How to master an exhausting marathon? Sustaining a long-term T-cell response during chronic infection requires both down-modulation of effector function and the maintenance of proliferative potential. In response to this challenge, CD8+ T cells can acquire metabolically distinct differentiation stages. While fast running individuals show superior effector function, they are being sacrificed on their way to the finish line. Others, however, run at a sustainable pace in order to retain their sprint potential. These metabolically adapted and rather fit individuals, termed precursors of exhausted T cells (Tpex), are characterized by a steady self-renewing capacity. Their proliferation pace is sustainable, yet there is potential for the rapid metabolic transition needed for effector sprints. This metabolic plasticity is required to continuously replenish terminal exhausted effector T cells (Tex) and is therefore a critical aspect for the prolonged T-cell response during chronic infection. Gabriel *et al.*[14] discovered that metabolic plasticity and the function of exhausted T cells is finely balanced via the TGF-β–mTOR signaling axis.

retain their metabolic fitness throughout the infection, whereas Id3− terminally exhausted cells lost most of their metabolic capacity as the infection progressed.

In search of a "switch" that regulates metabolic fitness in exhausted CD8+ T cells, Gabriel *et al.* hypothesized a role for mTOR, a master regulator of cellular metabolism.[16] By reanalyzing a previously generated sequencing data set[8] they revealed a transient downregulation of the hallmark genes involved in mTOR metabolism in Tpex early after infection. This correlated with a transient suppression of mTOR activity in the precursor population, as opposed to their terminally exhausted counterparts, at this early time point. Of note, while mTORC1 signaling could be activated in Tpex even at later stages of the infection, Tex showed an impaired ability to activate the pathway. Additionally, mTOR suppression by the mTORC1 inhibitor rapamycin increased metabolic fitness in effector T cells generated *in vitro*, confirming that restraining mTOR function is important for maintaining mitochondrial metabolism in CD8+ T cells.

These data are in line with *in vivo* observations showing that rapamycin is capable of restoring memory formation.[17] In chronic infection, rapamycin treatment caused a massive population shift towards the Id3+Tim3− precursor population: the transient inhibition of mTORC1 via rapamycin administered in an early time window (0–4 days) after chronic infection boosted the proliferation of exhausted T cells that produced high amounts of IFNγ at later stages of the infection.[14] Interestingly, the authors revealed a possible therapeutic potential of targeting this pathway: this early treatment with rapamycin, combined with a late checkpoint inhibition via anti-PD-L1 blockade, results in further expansion of CX3CR1+ Tex cells with superior viral control.

What remained a missing puzzle piece was the identification of an endogenous signal that regulates cellular metabolism in $CD8^+$ T cells in the course of a chronic infection; for this too, Gabriel *et al*. provide an answer. The cytokine TGF-β was a promising candidate to look at because TGF-β had previously been shown to dampen mTOR activity in NK cells[18] and to maintain T cell memory.[19, 20] Interestingly, an analysis of their sequencing data set revealed a significant enrichment of TGF-β signaling in Tpex compared with Tex. Using TGF-β knockout and overexpression mouse models, they could correlate TGF-β signaling capacity with the magnitude of mTOR signaling and found that TGF-β signaling was directly responsible for suppressing mTOR activity in T cells during chronic infection. By altering the magnitude of TGF-β signaling in $CD8^+$ T cells it was possible to fine-tune the development of Tex: while depletion of TGF-β signaling boosted the effector response, constitutively active TGF-β signaling enriched Tpex and inhibited the effector response by shifting the progeny of Tex to terminally differentiated $CD101^+$ cells. This example convincingly demonstrates how the external factor TGF-β can alter cellular metabolism by suppressing mTOR and can translate into the essential attenuation of effector function needed in chronically infected mice.

To illustrate the finding made by Gabriel *et al*., Figure 1 depicts how TGF-β directly interferes with the metabolism of exhausted T cells: by dampening the activity of the kinase mTOR, TGF-β preserves cellular metabolism and allows Tpex to "run" at a sustainable pace. In contrast, unleashing mTOR activity allows fast proliferation or a "sprint" accompanied by high effector function followed by premature exhaustion. Thus, using a combination of transgenic mouse models, RNA sequencing and *in vitro* and *ex vivo* T cell metabolic profiling, the authors identify TGF-β signaling and the kinase mTOR as master regulators of metabolic fitness in exhausted T cells and link their activity to T-cell differentiation and function in chronic infection.

Altogether, the findings presented by Gabriel *et al*. significantly expand our current view of the population dynamics of T cells in chronic infection. While it is established that chronic infection produces the aforementioned sub-populations of exhausted T cells, Gabriel *et al*. have now added insight into the metabolic pathways that contribute to their differentiation. However, it remains to be identified which environmental signals, besides TGF-β, influence Tpex to take the metabolic exit (Figure 1), to differentiate and unleash the effector response.

ACKNOWLEDGMENTS

AMS is a Klaus Tschira Boost Fellow administered by the Klaus Tschira Foundation and the German Scholar Organization, who is further supported by the DFG. DZ is an ERC and DFG funded principal investigator and Full Professor in immunology and infection biology. The figure was created with BioRender.com. Open access funding was enabled and organized by ProjektDEAL.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Anna M Schulz: Conceptualization; Writing – original draft; Writing – review & editing. **Dietmar Zehn:** Conceptualization; Writing – original draft; Writing – review & editing.

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