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Mitochondrial dysfunction defines T cell exhaustion

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When T cells are exposed to continuous antigen stimulation, they become exhausted. Here, we preview findings from Scharping et al. (2021), who have illuminated the molecular mechanism by which the persistent antigen stimulation and severe hypoxic conditions in the intratumoral environment drive T cell exhaustion, losing their cytotoxic function and anticancer effects.

$CD8^+$ T cells recognize and eliminate infected or malignant cells. However, after long exposure to the stimulus, $CD8^+$ T cells stop proliferating and lose their effector capacity, compromising the protective immune response. Persistent antigen stimulation give rise to different types of dysfunctional T cells including exhausted and senescent T cells. Both exhausted and senescent T cells present similar features, including impaired TCR-driven proliferation and expression of terminal differentiation markers. However, whereas exhausted T cells are characterized by reduced secretion of effector cytokines, such as IFN-γ and TNF-α, senescent T cells are characterized by persistent secretion of these inflammatory cytokines. Understanding the molecular mechanism that drives the acquisition of these dysfunctional, and sometimes even detrimental, states is essential to invigorate immunity.

Three different articles recently published in *Nature Immunology* from Thompson, Ho, and Delgoffe have dissected how intratumoral conditions, including hypoxia and persistent antigen exposure, induce the exhaustion of tumor infiltrating lymphocytes (TILs) (Scharping et al., 2021; Vardhana et al., 2020; Yu et al., 2020). In particular, Scharping et al. found that exhausted TILs presented a higher hypoxic state and mitochondrial stress than effector $CD8^+$ T cells (Scharping et al., 2021). Upon exhaustion conditions, T cells progressively acquire inhibitory molecule expression and their secretion properties are reduced, becoming progenitor exhausted cells. When the exhaustion environment is maintained, these progenitor exhausted T cells finally differentiate into terminally exhausted T cells, which present higher levels of inhibitory molecules and null response. To understand the causality between mitochondrial dysfunction and exhaustion, the authors developed an *in vitro* system to mimic T cell exhaustion by combining hypoxia and persistent TCR stimulation. Under these conditions, $CD8^+$ T cells recapitulated *in vivo* exhaustion features like expression of the transcriptional regulator Tox, increased expression of inhibitory receptors (PD-1, TIM-3, and LAG-3), and impaired cytokine production (IFN-γ and TNF-α) and cytotoxic activity.

Remarkably, hypoxia-inducible factor 1α was dispensable for the exhaustion phenotype. Instead, Blimp1, a transcriptional repressor and a critical regulator of T cell homeostasis, was required. Removing Blimp1 in tumor terminally exhausted T cells was sufficient to restore TIL functional roles *in vivo* and *in vitro*. Blimp1 overexpression reduced PGC1-α transcription, a transcriptional coactivator controlling mitochondrial biogenesis and antioxidant response. Previously, the authors had shown that overexpression of PGC1-α mitigates the exhaustion phenotype by boosting mitochondrial biogenesis (Scharping et al., 2016). Now, they extend these observations describing the contribution of PGC1-α as regulator of mitochondrial reactive oxygen species (mtROS) during T cell exhaustion. The researchers used low doses of chemical inhibitors targeting the electron transport chain (ETC) complexes I (rotenone) and

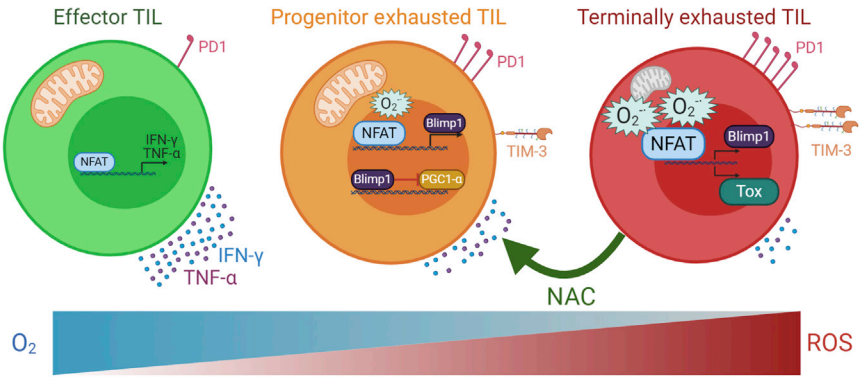

Figure 1. Hypoxia and chronic antigen exposure determine the exhaustion of TILs
The combination of hypoxia and persistent antigen stimulation in the tumor microenvironment damages mitochondria in TILs, triggering ROS production. The accumulation of ROS induces the nuclear translocation of NFAT and promotes an exhaustion-associated genetic program regulated by Blimp1 and TOX that terminally exhausts T cells. Blimp1 represses mitochondrial biogenesis and antioxidant response by downregulating PGC1-α. Notably, scavenging ROS with NAC treatment ameliorates some of exhaustion characteristics, reversing the terminally exhausted TILs to a progenitor exhausted state (created with BioRender.com).

III (antimycin A). Importantly, only the inhibition of complex III at these doses induced mtROS and T cell exhaustion. Treatment with antimycin A uncouples ETC at complex III, and then the reverse electron transport generates mtROS at complex I. Supporting a key role for mtROS as drivers of the exhausted phenotype, the authors found that inhibition of complex I, or the simultaneous inhibition of complexes I and III, neither produced mtROS nor drove T cell exhaustion. In addition, ROS scavenging by N-acetylcysteine (NAC) rescued exhaustion in T cells both *in vivo* and *in vitro*. This is in agreement with previous work from Thompson's lab that used NAC to restore the proliferation and self-renewal capacity of chronically stimulated T cells, improving antitumor immunity (Vardhana et al., 2020). According to the role of ROS in the activation of nuclear factor of activated T cells (NFAT) signaling, antimycin A-treated cells induced NFAT nuclear translocation, promoting Blimp1 and Tox expression. Altogether, the authors proposed that persistent activation of T cells under hypoxic conditions induces a mitochondrial stress that increases ROS levels, chronic signaling, and reinforcement of the exhaustion's transcriptional machinery (Figure 1). Treatments that allow escape from this feedback loop could rescue terminally exhausted T cells.

Preventing the chronic stimulation of T cells in tumors seems a difficult task. Fortunately, alleviating mtROS or hypoxia could be a feasible strategy. The authors checked both approaches with promising results. Overexpression of Gpx1, a glutathione peroxidase, made T cells resistant to ROS accumulation and exhaustion. On the other hand, ameliorating hypoxia in tumors by pharmacological (low doses of axitinib that inhibit aberrant tumor vascularization) or genetic (complex I depletion in tumor cells, which reduces oxygen consumption) approaches allowed TILs to retain effector functionality. Remarkably, even terminally exhausted TILs displayed less exhaustion markers upon these treatments. In line with these results, findings from Ho's lab indicate that treatment with NAD^+ precursors decreases mtROS levels, restoring mitochondrial fitness and increasing the antitumor function of TILs (Yu et al., 2020). Additionally, the effect of the treatment with axitinib or NAD^+ precursors can be improved by combination with checkpoint inhibitors (αPD-1).

Altogether, the novel discoveries from Thompson's, Ho's, and Delgoffe's labs place the loss of mitochondrial fitness as an essential player in the acquisition of the exhaustion phenotype by TILs. Their findings reveal new encouraging approaches to reverse T cell exhaustion by alleviating mitochondrial stress, opening a promising door for the future of tumor immunotherapy.

In line with these observations, exhausted $CD8^+$ T cells generated due to chronic infections present mitochondrial impairment, and mtROS alleviation with mitochondria-targeted antioxidants is sufficient to boost T cell function and improve the antiviral response (Fisicaro et al., 2017). Importantly, novel antiviral therapies have allowed researchers to investigate the fate of exhausted T cells after the removal of the antigen. Upon elimination of the persistent stimulus, terminally exhausted T cells disappear, whereas progenitor exhausted $CD8^+$ T cells persist, bearing a molecular scar of exhaustion (Hensel et al., 2021).

Exhausted T cells, together with senescent T cells, are also accumulated in lymphoid and non-lymphoid organs, such as the spleen, the lungs, and the liver, during healthy aging (Elyahu et al., 2019; Mogilenko et al., 2021). Results from our lab suggest that a dramatic mitochondrial failure due to mitochondrial transcriptional factor A (Tfam) depletion causes a dysfunctional state in T cells that fits with an exhausted or a senescent phenotype. Tfam deletion in T cells causes a severe mitochondrial DNA loss that impairs TCR-driven proliferation and immune effector functions (Baixauli et al., 2015; Desdín-Micó et al., 2020). However, as these T cells present a persistent production of IFN-γ and TNF-α, they likely resemble senescent rather than exhausted T cells. Due to this persistent production of inflammatory cytokines, senescent T cells contribute to inflammaging and tissue damage, thus accelerating biological aging (Desdín-Micó et al., 2020). In contrast, exhausted T cells, although ineffective in fighting tumors or infections, are considered to have less capacity to damage tissue due to their impaired secretion of classical effector cytokines such as IFN-γ or granzyme B. However, they produce high amounts of granzyme K, a cytokine that aggravates the senescence-associated secretory phenotype in bystander cells, contributing to inflammaging (Mogilenko et al., 2021).

More research is needed to delve into the differential pathways defining senescence and exhaustion in T cells. Although both states present mitochondrial dysfunction, impaired proliferation, and expression of terminal differentiation markers, senescent T cells become highly inflammatory whereas exhausted T cells shut down IFN-γ and TNF-α responses.

A remaining open question is whether T cells could shift from an exhausted to a senescent phenotype or vice versa. There is no doubt that modulation of the senescent or exhaustion programs into a fully functional state could open immense opportunities, not only in anti-tumor therapies, but also for preventing age-related immunosenescence.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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