Journal of the National Cancer Center 5 (2025) 105–107

Contents lists available at ScienceDirect

Journal of the National Cancer Center

journal homepage: www.elsevier.com/locate/jncc

Commentary

Ammonia-induced lysosomal and mitochondrial damage: a novel perspective on T cell-based cancer immunotherapy☆

Jianqiao Shentu[1,2,*], Hening Xu[1,2], Feng Zhu[1,2,*]

[1] Key Laboratory of Novel Targets and Drug Study for Neural Repair of Zhejiang Province, School of Medicine, Hangzhou City University, Hangzhou, China
[2] Department of Clinical Medicine, Hangzhou City University, Hangzhou, China

Metabolic reprogramming is a critical process in the activation and function of immune cells, wherein changes in metabolites and enzymes regulate the phenotype and function of immune cells by modulating energy metabolic pathways and signaling cascades.[1] In the realm of cancer immunotherapy, metabolic interventions in immune cells have demonstrated potential to enhance therapeutic efficacy. For instance, Minogue et al.[2] found that glutaric acid from tryptophan metabolism can modulate the antitumor T cell response by affecting pyruvate metabolism and α-ketoglutarate-dependent dioxygenases. The adjustment of glutaric acid levels in $CD8^+$ T cells has been shown to promote central memory T cell development, reduce exhaustion markers, and enhance cell death.

Recently, the research team led by Professor Huang Bo has elucidated a novel mechanism of regulated cell death associated with ammonia, a toxic byproduct of amino acid metabolism, which they have termed "ammonia death".[3] The study revealed that in long-term activated effector $CD8^+$ T cells ($CD8^+$ T_{eff} cells), a substantial amount of ammonia, generated by mitochondrial glutaminolysis, was released into the cytoplasm and subsequently enters lysosomes via the ammonium transporter Rhesus C glycoprotein (RHCG). This process results in a decline in lysosomal acidity and consequent impaired lysosomal function. When the lysosomal capacity to neutralize ammonia becomes saturated, ammonia accumulates in the mitochondria, inducing mitochondrial damage. This ultimately results in cell death and impairs the cytotoxic function of $CD8^+$ T_{eff} cells (Fig. 1). Ammonia-induced cell death, characterized by lysosomal alkalization and mitochondrial swelling, stands apart from apoptosis (marked by cell shrinkage, nuclear condensation, and caspase activation[4]), ferroptosis (driven by iron-dependent lipid peroxidation and ROS accumulation[5]), and pyroptosis (characterized by inflammatory cell swelling and cytokine release[6]). As elucidated by Ma et al., inhibiting ferroptosis in $CD8^+$ T cells augments their anti-tumor capabilities.[7] Similarly, cell death induced by ammonia demonstrates considerable potential in the domain of cancer therapy.

1. Discovery of ammonia-induced cell death in $CD8^+$ T_{eff} cells

Upon antigen stimulation, $CD8^+$ T cells become activated, undergo clonal expansion, and subsequently contract through cell death. Professor Huang's team investigated the role of ammonia in the demise of $CD8^+$ T_{eff} cells. In their experiments, $CD45.2^+$ mice were infused with $CD45.1^+$ OT-I T cells and subsequently infected with *Listeria monocytogenes* expressing ovalbumin. *In vitro* experiments confirmed that $CD8^+$ OT-I T_{eff} cells began to accumulate ammonia and undergo cell death by the 10th day. It was also discovered that carbamoylphosphate synthase 1 (CPS1) is absent in $CD8^+$ T_{eff} cells. However, its forced overexpression increased the production of urea cycle metabolites and urea, thereby rescuing $CD8^+$ T_{eff} cells from death both *in vivo* and *in vitro*. The addition of exogenous ammonia induced $CD8^+$ T_{eff} cell death, whereas treating infected mice with 4-phenylbutyrate (4-PBA) resulted in decreased ammonia levels and increased numbers of $CD8^+$ OT-I T_{eff} cells. Notably, inhibitors of non-caspase-dependent pathways, as well as ferroptosis and necroptosis inhibitors, had no effect on ammonia levels or cell death prevention. The dying $CD8^+$ T_{eff} cells exhibited unique cytoplasmic vacuoles, and cells activated late or treated with ammonia showed reduced ATP generation, which suggests that ammonia accumulation induces a unique form of cell death in $CD8^+$ T cells.

2. Molecular mechanisms

Given that mitochondrial glutaminase-1 (GLS1) catalyzes the conversion of intracellular glutamine to glutamate and ammonia, researchers administered GLS1 inhibitors JHU083 or CB839 to mice. These inhibitors significantly reduced intracellular ammonia levels and enhanced the number of cells, survival time, and effector factor expression in the spleen. These findings revealed that ammonia accumulation in long-term activated $CD8^+$ T_{eff} cells triggers apoptosis via a novel mechanism, with the core source being the decomposition of glutamine in the mitochondria.

Ammonia, with a pH of 9.3, can combine with protons to form ammonium ions, and lysosomes act as neutralizers to handle this weakly alkaline substance. Nitrogen tracing indicated that lysosomal ammonia primarily originates from the mitochondria, and inhibiting GLS1 reduces lysosomal ammonia levels. As ammonia levels rise, the lysosomal pH increases from 4.5 to 6.2, leading to impaired function, decreased enzyme activity, and reduced membrane permeability. Knocking down RHCG

☆ Comment on: Zhang H, Liu J, Yuan W, et al. Ammonia-induced lysosomal and mitochondrial damage causes cell death of effector CD8(+) T cells. *Nat Cell Biol.* 2024;26(11):1892–1902. doi:10.1038/s41556-024-01503-x.

* Corresponding authors.
E-mail addresses: Shentujq@stu.hzcu.edu.cn (J. Shentu), zhuf@hzcu.edu.cn (F. Zhu).

https://doi.org/10.1016/j.jncc.2025.01.004
Received 5 November 2024; Received in revised form 23 January 2025; Accepted 24 January 2025

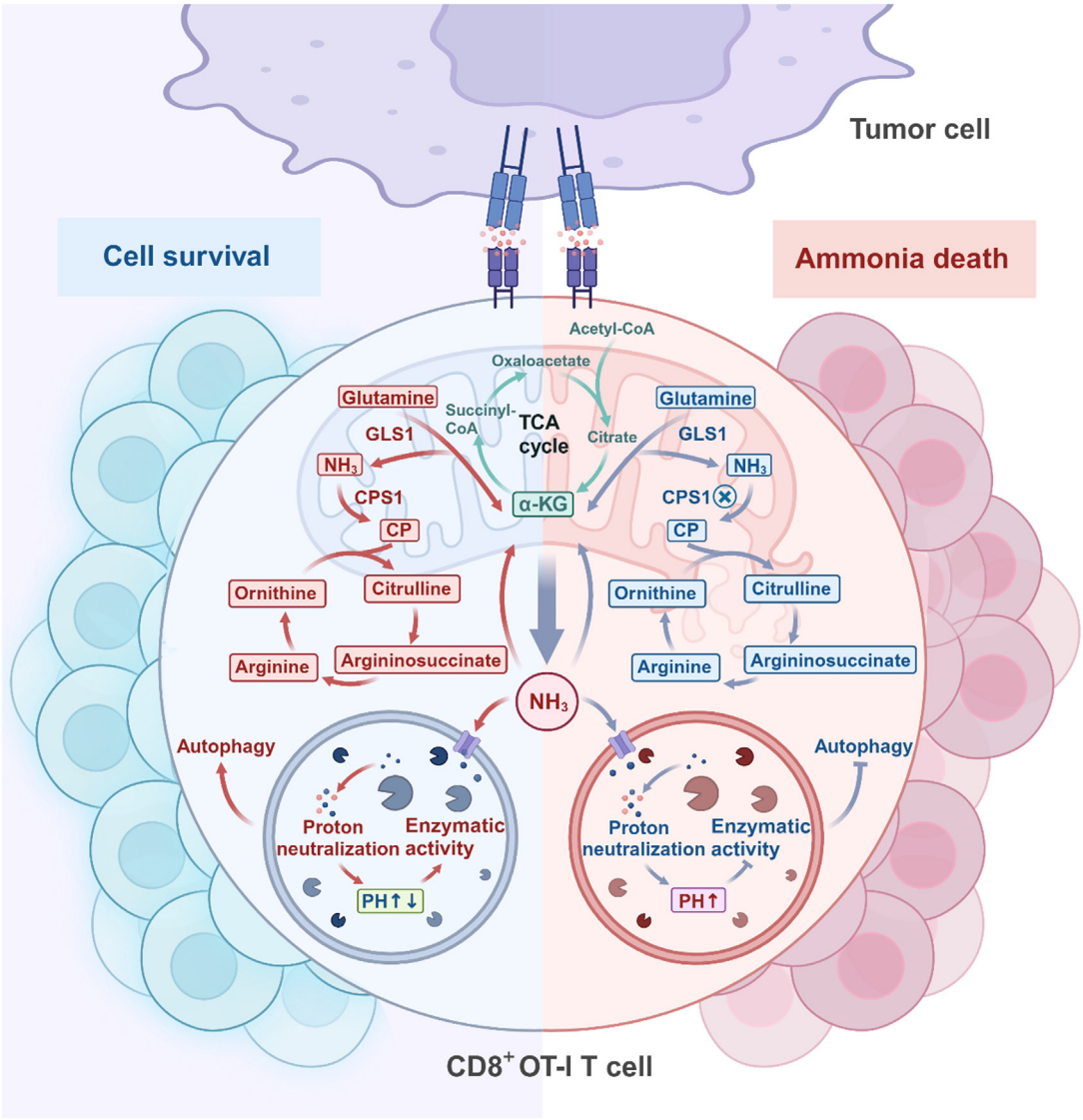

Fig. 1. Schematic illustration of the mechanism underlying ammonia-induced cell death in effector CD8$^+$ T cells. The research team led by Professor Huang, using CD8$^+$ OT-I T cells, elucidated the key mechanism of "ammonia death" in effector CD8$^+$ T cells (CD8$^+$ T_{eff} cells), which involves the accumulation of intracellular ammonia and its detrimental effects on lysosomal and mitochondrial function. In chronically activated CD8$^+$ T_{eff} cells, GLS1 catalyzes the production of α-KG and ammonia. α-KG enters the tricarboxylic acid cycle and participates in energy metabolism, whereas ammonia enters the urea cycle. However, due to the absence of CPS1 expression in CD8$^+$ T_{eff} cells, ammonia cannot be efficiently excreted by the urea cycle. The excess ammonia is then transported into lysosomes via the ammonia transporter RHCG. The influx of excess ammonia leads to the neutralization of lysosomes, decreased acidity, reduced enzyme activity, and impaired lysosomal function, resulting in lysosomal dysfunction and autophagy inhibition. After lysosomal saturation, ammonia backflows into mitochondria, where it accumulates, triggering a reduction in mitochondrial membrane potential, a decrease in DNA copy number, and the disruption of mitochondrial membrane and cristome structures. Damaged mitochondria cannot be removed by lysosome-mediated autophagy, resulting in cell death and impaired cytotoxic function of CD8$^+$ T_{eff} cells. Created with BioRender.com. α-KG, α-ketoglutarate; CD8$^+$ T_{eff} cells, effector CD8$^+$ T cells; CP, carbamoyl phosphate; CPS1, carbamoyl phosphate synthetase 1; GLS1, glutaminase-1; pH, pondus hydrogenii; RHCG, Rhesus C glycoprotein, TCA cycle, tricarboxylic acid cycle.

reduces ammonia entry into lysosomes and increases mitochondrial ammonia levels, whereas overexpressing RHCG increases lysosomal ammonia levels, further disrupting lysosomes and causing cell death. Studies on the impact of ammonia on the mitochondria of long-term activated CD8$^+$ T_{eff} cells found that high lysosomal ammonium concentrations prevent further uptake. Consequently, ammonia transported from the mitochondria to the cytoplasm refluxes back into the mitochondria. This causes further accumulation of ammonia in the mitochondria, leading to mitochondrial damage.

Further studies revealed no changes in the expression of autophagy markers, including PINK1, PARK2, and LC3, indicating that ammonia-mediated mitochondrial damage is not regulated by mitophagy. However, treatment with C381, a small molecule targeting vacuolar ATPase to reduce lysosomal pH, can restore the impaired autophagic flux, and C381 treatment reduces the death of activated CD8$^+$ T_{eff}. This further supports the critical role of increased lysosomal pH in ammonia-induced cell death, which may be a hallmark of this form of cell death.

3. Therapeutic application prospects

Researchers evaluated the impact of ammonia blockade on adoptive T-cell therapy. CD8$^+$ T_{eff} cells were adoptively transferred into B16 melanoma-bearing mice. Upon dissection, it was found that between days 4 and 10, tumor-infiltrating CD8$^+$ T cells exhibited significant ammonia accumulation and increased lysosomal pH. However, in CD8$^+$ OT-I T_{eff} cells, GLS1 knockdown or CPS1 overexpression, akin to treat-

ment with C381 or DON, reduced tumor growth, extended survival, and enhanced the persistence of OT-I cells in the tumor microenvironment. This suggests that blocking "ammonia death" may be a critical factor in enhancing the efficacy of adoptive T-cell therapy.

4. Discussion and conclusion

Professor Huang Bo's team has innovatively revealed a novel cell death mode closely related to ammonia metabolism, providing a new and important explanation for the rapid demise of $CD8^+$ T_{eff} cells.[3] This discovery not only opens a new perspective for understanding the role of ammonia in cellular physiology and pathology but also brings new possibilities for the development of novel therapeutic strategies, especially in the field of T cell-based cancer immunotherapy.

In the realm of translational medicine, particularly in adoptive T-cell therapy, the detrimental effects of ammonia accumulation on $CD8^+$ T_{eff} cells can be alleviated. Prior to treatment, gene editing can be employed to regulate the expression of CPS1 in transplanted $CD8^+$ T_{eff} cells, thereby preserving their function, combating ammonia accumulation, and enhancing their survival rates and anti-tumor activity. Concurrently, the development of small-molecule compounds that reduce ammonia levels can create a more favorable microenvironment. At present, some ammonia scavengers, such as sodium benzoate, sodium phenylbutyrate, and sodium glycerin phenylbutyrate, have been used in clinical or experimental work to reduce ammonia levels in the body, but their efficacy and safety need to be further evaluated. Additionally, identifying reliable prognostic biomarkers is crucial for optimizing cancer treatment strategies, especially in the era of personalized medicine where immunotherapy has emerged as a novel tumor intervention.[8,9] Integrating clinical testing with a monitoring system to regularly assess ammonia levels and functional indicators of $CD8^+$ T_{eff} cells in patient samples, and adjusting treatment plans accordingly with ammonia-regulating drugs or relevant interventions, would be a prudent approach to optimize therapeutic outcomes and patient prognosis.

Furthermore, the implications of this discovery extend to research on other immune cell types.[10] For $CD4^+$ T cells, investigations should focus on how ammonia accumulation affects their differentiation, proliferation, and immune regulatory functions. For instance, in models of autoimmune diseases, studying the interference of ammonia on the differentiation of their subtypes and cytokine secretion could lead to the development of ammonia metabolism modulation strategies. In the case of macrophages, research should explore the impact of ammonia on their polarization. When ammonia-induced abnormal polarization affects tumor immunity, interventions targeting ammonia metabolism can help reshape their function to aid in tumor immunotherapy. Moreover, for natural killer cells, the exploration of changes in their activity, toxicity, and target cell recognition capabilities under ammonia-rich conditions is crucial. If ammonia inhibits their function, regulating ammonia metabolism could enhance their anti-tumor and antiviral capabilities.

Despite significant findings on "ammonia death," the field is still in its nascent stages of development. To further elucidate the mechanisms of "ammonia death" and its potential pathophysiological functions for clinical translation, future research should explore the following aspects: (1) The transport and reflux pathways of ammonia in various organelles remain unclear, and further labeling experiments could validate the reflux pathway and mechanism of ammonia from lysosomes to mitochondria; (2) Additional experiments, such as flow cytometry analysis or fluorescence imaging technology, could further investigate the specific mechanisms by which ammonia disrupts mitochondrial membrane potential and morphology; (3) It remains to be explored whether ammonia affects other subcellular structures, such as the Golgi apparatus (which produces primary lysosomes), to induce T cell death; and (4) The process of lysosomal pH elevation due to ammonia accumulation and its potential feedback effects on mitochondria remain unknown.

In summary, by thoroughly investigating the role of ammonia in the life cycle of $CD8^+$ T_{eff} cells, this research not only broadens our understanding of immune cell metabolic mechanisms but also provides robust theoretical support for the precise regulation of ammonia levels, laying a crucial foundation for future biomedical research and clinical applications, and offering valuable practical guidance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by Zhejiang Provincial Natural Science Foundation of China (grant number: LGD22H090003).

Author contributions

J.S. analyzed the literature and wrote the manuscript. J.S. and H.X. drafted the figure. J.S. and F.Z. conceived the idea. F.Z. reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

References

1. Reina-Campos M, Scharping NE, Goldrath AW. CD8(+) T cell metabolism in infection and cancer. *Nat Rev Immunol*. 2021;21(11):718–738. doi:10.1038/s41577-021-00537-8.
2. Minogue E, Cunha PP, Wadsworth BJ, et al. Glutarate regulates T cell metabolism and anti-tumour immunity. *Nat Metab*. 2023;5(10):1747–1764. doi:10.1038/s42255-023-00855-2.
3. Zhang H, Liu J, Yuan W, et al. Ammonia-induced lysosomal and mitochondrial damage causes cell death of effector CD8(+) T cells. *Nat Cell Biol*. 2024;26(11):1892–1902. doi:10.1038/s41556-024-01503-x.
4. Ketelut-Carneiro N, Fitzgerald KA. Apoptosis, pyroptosis, and necroptosis-oh my! the many ways a cell can die. *J Mol Biol*. 2022;434(4):167378. doi:10.1016/j.jmb.2021.167378.
5. Stockwell BR. Ferroptosis turns 10: emerging mechanisms, physiological functions, and therapeutic applications. *Cell*. 2022;185(14):2401–2421. doi:10.1016/j.cell.2022.06.003.
6. Cai H, Zhang J, Xu H, et al. ALOX5 drives the pyroptosis of CD4(+) T cells and tissue inflammation in rheumatoid arthritis. *Sci Signal*. 2024;17(825):eadh1178. doi:10.1126/scisignal.adh1178.
7. Ma X, Xiao L, Liu L, et al. CD36-mediated ferroptosis dampens intratumoral CD8(+) T cell effector function and impairs their antitumor ability. *Cell Metab*. 2021;33(5):1001–1012.e5. doi:10.1016/j.cmet.2021.02.015.
8. Sahin TK, Ayasun R, Rizzo A, Guven DC. Prognostic value of neutrophil-to-eosinophil ratio (NER) in cancer: a systematic review and meta-analysis. *Cancers (Basel)*. 2024;16(21):3689. doi:10.3390/cancers16213689.
9. Rizzo A, Santoni M, Mollica V, et al. Peripheral neuropathy and headache in cancer patients treated with immunotherapy and immuno-oncology combinations: the MOUSEION-02 study. *Expert Opin Drug Metab Toxicol*. 2021;17(12):1455–1466. doi:10.1080/17425255.2021.2029405.
10. Di Federico A, Mosca M, Pagani R, et al. Immunotherapy in pancreatic cancer: why do we keep failing? A focus on tumor immune microenvironment, predictive biomarkers and treatment outcomes. *Cancers (Basel)*. 2022;14(10):2429. doi:10.3390/cancers14102429.