EDITORIAL

Ammonia death: a novel potential strategy to augment immunotherapy in cancer

The efficacy of immunotherapy is diminished by the low survival rate of effector CD8 + T cells after they have exerted their antitumor effects. Recent studies indicate that ammonia, generated from glutamine metabolism, accumulates in effector CD8 + T cells and triggers their apoptosis. These findings offer a comprehensive mechanistic understanding of effector T-cell mortality from a metabolic viewpoint, presenting novel opportunities for improving T-cell-mediated anticancer treatments.

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INTRODUCTION

Immunotherapy has emerged as a revolutionary breakthrough in cancer treatment in recent years, significantly improving the prognosis of various tumors. However, optimizing T-cell survival and function remains one of the key challenges in enhancing the efficacy of immunotherapy [1]. A significant obstacle is T-cell exhaustion, wherein antitumor T cells diminish their efficacy within the hostile tumor milieu, frequently resulting in immune evasion by the tumor [2]. A significant constraint is the reduced survival rate of effector CD8 + T cells following the eradication of antigen-presenting tumor cells. Upon completing their tumor-targeting function, these T cells undergo programmed cell death, diminishing the long-term efficacy of immunotherapy [3]. Consequently, improving the viability and efficacy of CD8 + T cells is a pressing concern in immunotherapy.

This issue of *Nature Cell Biology* features a study by Huafeng Zhang et al. [4] that identifies a novel form of T-cell death, referred to as 'ammonia-induced cell death,' which could significantly impact the field of immunotherapy. The research indicates that ammonia plays a critical role in the mortality of effector CD8 + T cells. Activated CD8 + T cells rapidly proliferate and undergo metabolic reprogramming during the immune response to satisfy their elevated energy requirements for antitumor activity. Glutamine metabolism is essential in this process, supplying carbon and nitrogen for biosynthesis and energy generation [5]. Nonetheless, the accelerated metabolism of glutamine produces ammonia as a metabolic byproduct. Upon activation, CD8 + T cells exhibit a gradual increase in intracellular ammonia levels, which ultimately results in cell death (Fig. 1). Previous studies primarily focused on traditional mechanisms of effector CD8 + T-cell death, such as programmed cell death and T-cell exhaustion. However, these investigations largely overlooked the role of metabolic factors in cell death, particularly the production and accumulation of ammonia within cells. Zhang and colleagues' research unveils the mechanisms of ammonia-induced cell death from a metabolic perspective, thereby providing a novel insight into the issue of effector T-cell survival. This discovery provides a new explanation for the swift decline of effector T cells after they fulfill their antitumor functions. This study diverges from previous research, which mainly attributed effector CD8 + T-cell death to apoptosis or exhaustion, by identifying ammonia as a significant metabolic factor contributing to their demise. Investigating the relationship between glutamine metabolism and ammonia-induced cell death provides opportunities for mitigating ammonia toxicity and improving the survival of effector T cells in the tumor microenvironment.

MECHANISMS AND IMPLICATIONS OF AMMONIA DEATH IN IMMUNOTHERAPY

The research examined the molecular mechanisms through which ammonia causes CD8 + T-cell death. The study's key finding indicates that ammonia induces toxicity through the impairment of two essential organelles: lysosomes and mitochondria. Lysosomes function to degrade and recycle cellular waste, thereby maintaining cellular homeostasis. The research demonstrated that ammonia directly compromises the lysosomal membranes of CD8 + T cells, resulting in the leakage of degradative enzymes into the cytoplasm. Lysosomal damage is a critical event that initiates a series of detrimental intracellular reactions, ultimately resulting in cell death. The researchers identified that ammonia is transported into lysosomes through a specific transporter known as Rhesus glycoprotein C (RHCG). This study elucidates the molecular mechanism by which ammonia is transported into lysosomes and identifies a potential therapeutic target for inhibiting this process, thus safeguarding T cells from ammonia-induced toxicity. The study revealed that when lysosomes are unable to absorb ammonia, excess ammonia accumulates in other cellular regions, particularly in the mitochondria. Mitochondria, known as the cell's energy powerhouse, exhibit significant sensitivity to metabolic disruptions. The accumulation of ammonia within mitochondria results in dysfunction, which induces oxidative stress, diminishes membrane potential, and ultimately leads to cell death [6]. The dual mechanism by which ammonia induces damage to lysosomes and mitochondria underscores the complex toxic effects of ammonia on effector CD8 + T cells. Understanding the mechanisms by which ammonia disrupts lysosomal and mitochondrial functions enables researchers to investigate strategies for mitigating this damage and prolonging T-cell lifespan.

Autophagy is essential for the removal of damaged mitochondria in the context of ammonia-induced damage [7]. The study indicates that mitochondrial damage resulting from ammonia is

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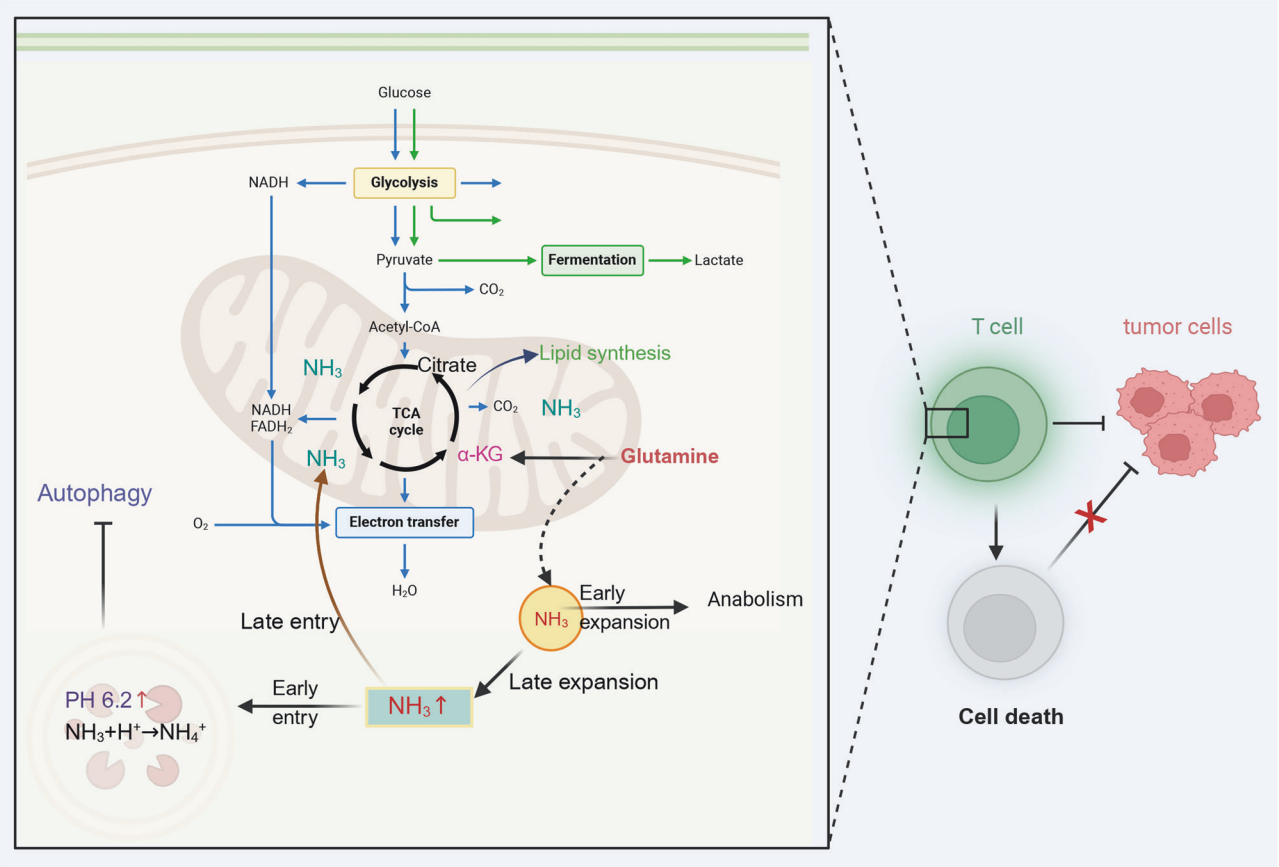

Fig. 1 Ammonia-induced T-cell death. In mitochondrial glutamine metabolism, ammonia (NH3) is produced and subsequently leaves the mitochondria to enter the lysosomes, where it reacts with H+ to form NH4 +. This process results in elevated lysosomal pH, thereby impairing lysosomal function. The excessive accumulation of NH3 in the cytoplasm inhibits its normal transport into lysosomes, leading to a reverse flow into the mitochondria, which causes mitochondrial damage. Damaged mitochondria are usually degraded through autophagy; however, an increased lysosomal pH hinders lysosomal enzymatic activity, which obstructs the removal of dysfunctional mitochondria. Damaged mitochondria accumulation subsequently induces effector T-cell death. *Created with BioRender.com*.

Table 1. Potential strategies and mechanisms of targeting ammonia-induced cell death in cancer immunotherapy.

Strategy	Mechanism	Potential benefits	Clinical relevance
Inhibiting ammonia accumulation	Blocking RHCG transport	Reducing lysosomal and mitochondrial damage	Prolonging T-cell lifespan
Enhancing autophagy	Promoting clearance of damaged mitochondria	Rescuing T cells from ammonia-induced apoptosis	Enhancing T-cell-mediated tumor clearance
Ammonia metabolism modulation	Targeting glutamine metabolism	Preventing excessive ammonia production	Increasing efficacy of adoptive T-cell therapies

excessively severe for autophagy to adequately eliminate, resulting in the accumulation of impaired mitochondria and worsening cellular dysfunction and mortality. The impairment of autophagy suggests that ammonia not only directly harms organelles but also diminishes the cell's capacity to repair such damage, thereby hastening cell death. This discovery introduces a novel therapeutic strategy: enhancing autophagy or expediting the removal of damaged organelles may rescue T cells from ammonia-induced apoptosis, thus augmenting the efficacy of immunotherapy. Immune "cold" tumors often exhibit poor responses to immunotherapy due to inadequate immune cell infiltration. Regulating ammonia metabolism may enhance T-cell survival within these microenvironments and increase their infiltration, thereby improving therapeutic efficacy against immune "cold" tumors. Future research could further explore the effects of ammonia metabolism modulation on different types of tumors, particularly those that respond poorly to conventional immunotherapies.

This study's primary significance is its potential for clinical translation. The study indicated that inhibiting ammonia-induced cell death can markedly improve the effectiveness of adoptive T-cell therapy. Currently, several ammonia-clearing agents, such as sodium benzoate, sodium phenylbutyrate, and glycerol phenylbutyrate, are utilized in clinical or experimental settings due to their efficacy in reducing ammonia levels in the body. Common side effects associated with these agents include mild allergic reactions, nausea, and abdominal pain. A comprehensive evaluation of the efficacy and safety of these products is essential for developing more effective treatment strategies for patients. In preclinical cancer models, researchers demonstrated that inhibiting ammonia accumulation or mitigating its toxic effects can prolong the survival of CD8 + T cells and improve their

functionality [8–10]. The findings indicate that targeting ammonia metabolism could serve as a new approach to enhance the durability and efficacy of cancer immunotherapy. This discovery provides potential solutions for a significant challenge in contemporary immunotherapies: the restricted longevity of effector T cells. Preventing ammonia-induced cell death may extend T-cell lifespan within the tumor microenvironment, leading to enhanced antitumor activity. This strategy may be integrated with current immunotherapies, including immune checkpoint inhibitors or CAR-T-cell therapy, to enhance synergistic effects and optimize patient outcomes (Table 1).

CHALLENGES AND FUTURE DIRECTIONS

This study indicates that ammonia generated from glutamine metabolism is a significant contributor to the mortality of effector CD8 + T cells; however, the specific regulatory mechanisms governing ammonia accumulation are not well understood. The study demonstrated that activated CD8 + T cells generate significant quantities of ammonia; however, the reasons for their inadequate clearance or processing of this substance necessitate additional research. Future research should investigate potential defects in ammonia clearance pathways, which may reveal organelle dysfunction or metabolic bottlenecks, offering insights into novel approaches to mitigate T-cell death. This study primarily examined effector CD8 + T cells; however, the immune system constitutes a complex network in which other immune cells, including CD4 + T cells, regulatory T cells, and natural killer cells, also contribute significantly to the tumor immune response. The applicability of these findings to other immune cell types and the exclusivity of ammonia-induced cell death to CD8 + T cells require further confirmation. Additionally, it is essential to ascertain the applicability of these findings across various cancer types and stages, necessitating further experimental validation. Despite notable advancements in vitro and in murine models, these systems fail to adequately mimic the intricacies of the human tumor microenvironment. Hypoxia, elevated lactate levels, and nutrient competition within the tumor microenvironment may affect ammonia metabolism and its detrimental impact on T cells. Translating these findings into clinical therapies presents a significant challenge. The therapeutic potential of inhibiting ammonia-induced T-cell death is promising; however, prolonged suppression of this process may lead to concerns regarding side effects. The programmed cell death of T cells is essential for maintaining immune balance; an excessive extension of effector T-cell lifespan can result in autoimmune diseases. Ammonia is a byproduct of normal cellular metabolism and plays a significant role not only in effector T cells but also in other normal and cancer cells. Therefore, it is crucial to ensure that therapeutic strategies targeting ammonia do not significantly impact the normal metabolism of these cells. Meanwhile, the rapid proliferation of cancer cells is accompanied by enhanced metabolic activity and ammonia production, suggesting that ammonia may play a role in cancer cell survival and tumor progression. Consequently, regulating ammonia metabolism could benefit immune cells while also potentially affecting cancer cells themselves. The long-term safety of these intervention strategies requires careful evaluation, and it is essential to identify the optimal therapeutic window to mitigate potential adverse effects.

Future research should further investigate the mechanisms of ammonia metabolism, potentially integrating these findings with other immunotherapeutic strategies to enhance the durability and efficacy of cancer treatments. Enhanced comprehension of cellular mechanisms for ammonia clearance and conversion, alongside the regulation of ammonia levels within the tumor microenvironment, may facilitate the development of novel therapeutic approaches aimed at mitigating ammonia's detrimental impact on effector T cells. Investigating the potential to protect T cells from ammonia toxicity through enhanced ammonia clearance capacity or optimized ammonia metabolic pathways is particularly important. The integration of ammonia-induced cell death with established immunotherapies, including CAR-T-cell therapy and immune checkpoint inhibitors, may produce synergistic outcomes. Additionally, the integration of ammonia metabolism inhibition with other strategies, including metabolic regulation or nutritional supplementation, merits investigation to improve the durability and effectiveness of adoptive T-cell therapies. Future research should investigate additional cancer types to ascertain whether ammonia-induced cell death is a universal occurrence or if certain cancer types exhibit greater susceptibility to ammonia accumulation. Further experimental and clinical validation is required to elucidate the differences between solid tumors and hematologic malignancies. Screening patients for metabolic profiles to identify those more susceptible to ammonia accumulation and designing targeted therapies can enhance the effectiveness of immunotherapy. This research direction aims to address current challenges in immunotherapy and may facilitate innovative breakthroughs in cancer treatment.

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AUTHOR CONTRIBUTIONS

ZL initiated the study and wrote the manuscript. All authors revised the manuscript and approved the final manuscript version to be published.

COMPETING INTERESTS

The authors declare no competing interests.