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Research Article

Lactate conditioning reprograms mucosal-associated invariant T cell metabolism boosting effector function

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Abstract

Mucosal-associated invariant T (MAIT) cells are unconventional T cells, which upon activation can display potent cytotoxic and cytokine-producing capabilities. Together, these features make MAIT cells promising candidates for cancer immunotherapy. In this study, we show that MAIT cells can be efficiently amplified in vitro, and these amplified MAIT cells are armed with potent anticancer functions, including the ability to produce significant amounts of effector molecules such as IFNγ and granzyme B. Excitingly, we demonstrate that MAIT cells can be redirected to potently kill cancerous cells using a clinically relevant bispecific monoclonal antibody. Furthermore, in an attempt to metabolically condition MAIT cells to improve function, we demonstrate that MAIT cells possess the molecular machinery to transport and metabolize lactate, an abundant metabolite within the solid tumor microenvironment. Activating MAIT cells in the presence of exogenous sodium lactate remodels their cellular metabolism, with a significant increase in mitochondrial metabolism. Functionally, this supports elevated production of effector molecules (IFNγ, granzymes A and B), leading to boosted engager mediated MAIT cell cytotoxicity. These data collectively show that MAIT cells can be pharmacologically directed to target cancer cells and in vitro conditioning using sodium lactate can enhance their anticancer capabilities through reprogrammed cellular metabolism. Our findings represent a novel strategy for a potential new adoptive cancer immunotherapy.

Keywords BiTEs, immunotherapy, lactate, mucosal associated invariant T cells

Introduction

Cancer immunotherapy has transformed oncology by harnessing the immune system's ability to recognize and eliminate malignant cells. T cell–based approaches, such as chimeric antigen receptor T cell therapy and bispecific monoclonal antibody engagers, have demonstrated remarkable success in hematological malignancies.[1,2] However, their efficacy in solid tumors remains limited due to immunosuppression within the tumor microenvironment (TME) and challenges in effectively targeting tumor cells.[3,4]

Mucosal-associated invariant T (MAIT) cells are an unconventional subset of T cells that recognize microbial-derived riboflavin metabolites presented by the highly conserved major histocompatibility complex–related molecule MR1.[5–7] This unique mechanism of antigen recognition enables MAIT cells to respond rapidly and potently to many bacterial infections, secreting high levels of effector cytokines and lytic molecules such as IFNγ.[8–10] In addition, MAIT cells have emerged as a promising candidate for cellular immunotherapy due to their innate-like properties such as rapid proliferation and cytokine production upon activation.[11–13] Recent evidence importantly indicates that MAIT cells exhibit strong antitumor potential through their cytotoxic abilities, combined with their capacity to activate other anticancer immune cells and non–major histocompatibility complex restriction.[14–16] Taken together, this makes MAIT cells potentially promising candidates for cancer immunotherapy that are currently underexplored.

A significant challenge for immune cells in the TME is the metabolic competition arising from cancer cells.[3] Tumor cells engage in aerobic glycolysis, consuming large amounts of glucose and producing high lactate concentrations as a by-product.[17] This metabolic competition creates a hostile environment in which immune cells struggle to sustain their anticancer functions.[18–20] Our research team and others have shown that MAIT cells—like other effector T cells—depend heavily on glycolysis

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for their immune functions, indicating that limited glucose in the TME might hinder their antitumor effectiveness.[21–24] Yet, whether other metabolic pathways can support MAIT cell functions remains unclear.

While lactate has traditionally been viewed as a metabolic waste product, emerging evidence suggests that immune cells, such as CD8+ cells, can utilize lactate as an alternative energy source to maintain their effector functions in pH-neutral and healthy tissue environments.[25,26] Similarly, recent studies have demonstrated that metabolically rewiring CD8+ T cells by changing their culture conditions can boost their effectiveness as adoptive cellular therapies.[27–29] The extent to which MAIT cells can metabolize lactate and its impact on their functional responses is not known.

Here, we demonstrate that MAIT cells can be rapidly expanded in vitro with amplified cell display robust anticancer functions, which can be directed using a clinically relevant bispecific T cell engager. We also reveal that MAIT cells have the essential transporters and enzymes for lactate metabolism. When supplied with exogenous sodium lactate in vitro, MAIT cells undergo metabolic reprogramming, resulting in increased mitochondrial respiration and enhanced production of IFNγ, granzyme B, and granzyme A. Together, our study identifies lactate conditioning as a potential strategy to enhance MAIT cell antitumor properties that could potentially improve MAIT cell–based immunotherapy by enhancing mitochondrial metabolism leading to improved antitumor immunity.

Methods

Study cohorts and ethical approval

A total cohort of 20 healthy adult donors was recruited. Inclusion criteria included ability to give informed consent, 18 to 65 yr of age, body mass index <28 kg/m^2, and no current or recent (<2 wk) infection. Ethical approval was obtained from both St Vincent's University Medical Ethics Committee and Maynooth University Ethics Committee.

Preparation of peripheral blood mononuclear cells and flow cytometric analysis

Peripheral blood mononuclear cell **(**PBMC) samples were isolated, using SepMate isolation tubes, by density-centrifugation over Lymphoprep (both STEMCELL Technologies), from fresh human peripheral blood samples. PBMCs were either stored at −70 °C or used immediately for MAIT cell expansion using 5-ARU-MG and IL-2 as previously described.[30]

MAIT cell cytokine analysis

IL-2–expanded MAIT (1×10^6/mL) cells (with $>$85% purity) were activated utilizing anti-CD3/CD28 Dynabeads (Gibco), IL-12/IL-18 stimulation (both 50 ng/mL), or cocultured with HT29 cancer cells at a ratio of 5:1 MAIT cells to target cells, with or without the addition of cibistamab (200 nM) (HY-P99011M MCE), and in the absence or presence of sodium (L) lactate (45 mM) (L7022; Sigma-Aldrich) or vehicle control (phosphate-buffered saline [PBS]) for a duration of 18 h. Following the 18-h period, the culture supernatants were evaluated for IFNγ, granzyme B, or granzyme A levels using ELISAs performed as per the manufacturer's instructions (R&D Systems).

MAIT cell degranulation assay

PBMCs were thawed and rested before addition of either sodium (L) lactate (45 mM) or vehicle control (PBS). PBMCs were activated utilizing anti-CD3/CD28 Dynabeads, and after 30 min, protein transport inhibitor cocktail (Invitrogen) was added and culture continued for a further 2.5 h. MAIT cells were identified by flow cytometry with staining using specific surface monoclonal antibodies, namely CD3, CD161, and TCRVa7.2 (all Miltenyi Biotec), and degranulation assessed according to the percentage of MAIT cells expressing CD107a (Miltenyi Biotec). Cell populations were acquired using a Attune NXT flow cytometer and analyzed using FlowJo software version 10 (BD Biosciences). Results were expressed as a percentage of the parent population as indicated and determined using flow-minus-one and unstained controls.

MAIT cell cytotoxicity assays

IL-2–expanded MAIT cells ($>$85%) were cocultured with Calcein AM (BD Pharmingen)–labeled and 5-ARU-MG–pretreated K562 cells at a ratio of 3:1 MAIT cells to target cells in the absence or presence of sodium (L) lactate (45mM) or vehicle control (PBS). After 2.5 h of coculture, supernatant was analyzed using a BMG Clariostar multimode microplate reader to measure supernatant fluorescence at 485 nm excitation and 525 nm emission and percentage killing calculated as a proportion of maximum killing by Triton X.

IL-2–expanded MAIT cells ($>$85%) were pretreated with cibistamab (200 nM) or vehicle control (PBS) and then cocultured with CFSE (Sigma-Aldrich)-labeled HT29 cells at a ratio of 5:1 MAIT cells to target cells, with or without sodium (L) lactate (45 mM) or vehicle control (PBS), for a duration of 18 h. Cell viability was determined using Invitrogen Fixable Viability Dye (Near-IR), and cell populations were analyzed utilizing flow cytometric analysis.

MAIT cell proliferation analysis

Fresh PBMCs (2×10^6/mL) were stimulated with 5 ng/mL of 5-ARU-MG. After 24 h, the media was replaced with new culture media containing IL-2 at 6.8 ng/mL. On day 3, the media was again replaced with fresh culture media enriched with IL-2 (34 ng/mL) and containing sodium (L) lactate at 22.5 mM. On day 6, the media replacement was repeated with the same IL-2 and sodium (L) lactate concentrations. On day 7, the frequencies of MAIT cells were analyzed using flow cytometry.

Analysis of the lactate transport and metabolic machinery in MAIT cells

The identification of monocarboxylate transporters (SLC16A1, SLC16A3, SLC16A7) and lactate dehydrogenase enzyme (lactate dehydrogenase A [LDHA] and LDHB) in MAIT cells was based on in silico analysis of a published MAIT cell proteomic dataset[22] downloaded from PRIDE accession number PXD041544 (https://www.ebi.ac.uk/pride/archive/projects/PXD041544). Expression was confirmed using quantitative polymerase chain reaction, messenger RNA of IL-2–expanded MAIT cells (with or without stimulation with anti-CD3/CD28 Dynabeads) was extracted using TRIzol according to the manufacturer's protocol. Synthesis of complementary DNA was performed using qScript cDNA Synthesis kit (Quanta Bio). Quantitative polymerase chain reaction was performed using PerfeCTa SYBR Green FastMix, Reaction Mix (Green FastMix, ROXTM) (QuantaBio) and KiCqStart primer sets (Sigma-Aldrich). For studies investigating the impact of lactate transport/metabolism inhibition, IL-2–expanded MAIT cells (>85%) were activated utilizing anti-CD3/CD28 Dynabeads in the absence or presence of sodium (L) lactate (45 mM) (L7022; Sigma-Aldrich) or vehicle control (PBS) for a duration of 18 h in the absence or presence of the MCT1 inhibitor AZD3965 (10 nM) or the MCT4 inhibitor VB124 (1 μM) or the lactate dehydrogenase inhibitor GSK2837808A (10 nM). Following the 18-h period, the culture supernatants were evaluated by ELISA.

MAIT cell seahorse assay

IL-2–expanded MAIT cells (>85% purity) were activated with anti-CD3/CD28 TCR Dynabeads for 24 h, either with sodium (L) lactate or vehicle control (PBS). Metabolic analysis utilized the Seahorse Extracellular Flux Analyzer XFe96 (Agilent). MAIT cells were resuspended in RPMI phenol red–free media supplemented with glucose (10 mM), glutamine (2 mM), and pyruvate (1 mM) (all from Agilent) and then seeded onto a Cell-Tak (354240; Corning) coated microplate for adhesion. Mitochondrial and glycolytic respiration parameters were assessed by measuring OCR (pmol/min) and ECAR (mpH/min), respectively. The injections comprised oligomycin (1 μM), FCCP (1 μM), rotenone (1 μM), antimycin A (1 μM), and monensin (20 μM). All chemicals were sourced from Merck.

MAIT cell ATP assay

IL-2–expanded MAIT cells (1×10^6/mL) were stimulated using anti-CD3/CD28 TCR Dynabeads, with or without sodium lactate for 18 h. ATP levels were measured using a luminescence ATP assay kit (Abcam). Reagents in the kit were reconstituted as per manufacturer's instructions. A standard curve was also prepared as per the kit's instructions. MAIT cells were harvested and washed with PBS. A total of 100 μL of resuspended MAIT cells was added to a black-walled, clear-bottom plate. A total of 50 μL of detergent was added to each well and the plate was placed on an orbital shaker for 5 min at 600 to 700 rpm. A total of 50 μL of substrate solution was added and the plate was returned to the orbital shaker for 5 min at 600 to 700 rpm. The plate was then covered and placed in the dark for 10 min before luminescence was measured on a multimode plate reader (CLARIOstar).

Statistics

Statistical analysis was completed using GraphPad Prism 6 software (GraphPad Software). Data are expressed as SEM. Distribution was assessed using Shapiro-Wilk test. We determined differences between two groups using Student's *t* test (paired or unpaired) or Wilcoxon signed rank test where appropriate. Analysis across 3 or more groups was performed using 1-way analysis of variance with Tukey multiple comparisons test linear regression models as appropriate. The *P* values were expressed with significance set at <0.05.

Results

Human MAIT cells rapidly proliferate in vitro and are armed with potent effector functions

We first demonstrate that peripheral blood MAIT cells rapidly expand in vitro in response to cognate antigen stimulation and IL-2, potentially allowing the production of large cell quantities for adoptive cell therapy (Fig. 1A). Next, we investigated whether in vitro amplified MAIT cells maintained their functionality when stimulated with anti-CD3/CD28 Dynabeads (TCR), revealing strong secretion of IFNγ, granzyme A, and granzyme B (Fig. 1B–D). Importantly, we show that amplified MAIT cells can be directed using the clinically relevant bispecific T cell engager Cibistamab to release IFNγ, granzyme A, and granzyme B (Fig. 1E–H). Excitingly, we show that bispecific engager can drive increased rates of colorectal cancer (HT29) cell death by amplified MAIT cells (Fig. 1I).

MAIT cells express transporters and enzymes involved in lactate metabolism

Several recent studies have demonstrated that in vitro sodium lactate conditioning can boost CD8+ T cell responses.[28,29] Therefore, we interrogated our previously published dataset on MAIT cell proteomics,[22] to investigate the expression of key components of lactate metabolism. Here, in vitro IL-2–amplified MAIT cells express monocarboxylate transporters, which play a role in the transport of lactate. Notably, MCT1 (SLC16A1) levels are increased upon TCR stimulation, whereas MCT2 (SLC16A7) and MCT4 (SLC16A3) levels were unchanged (Fig. 2A–C). Additionally, we evaluated if MAIT cells produce the enzymes essential for lactate metabolism. At the transcript levels, messenger RNA levels of LDHA and LDHB were significantly increased upon TCR stimulation (Fig. 2F, G). However, interrogation of the proteomics dataset did not reveal significant changes in LDHA or LDHB upon stimulation (Fig. 2D, E). These data demonstrate that human MAIT cells express lactate transporters and the enzymes required for its metabolism.

Exogenous sodium lactate treatment remodels MAIT cell metabolism

Elevated lactate levels have been shown to occur in multiple pathophysiological conditions such as infection, rheumatoid arthritis, and cancer, in which it can modulate both innate and adaptive immunity.[25] Therefore, we subsequently explored whether introducing sodium lactate into our culture system

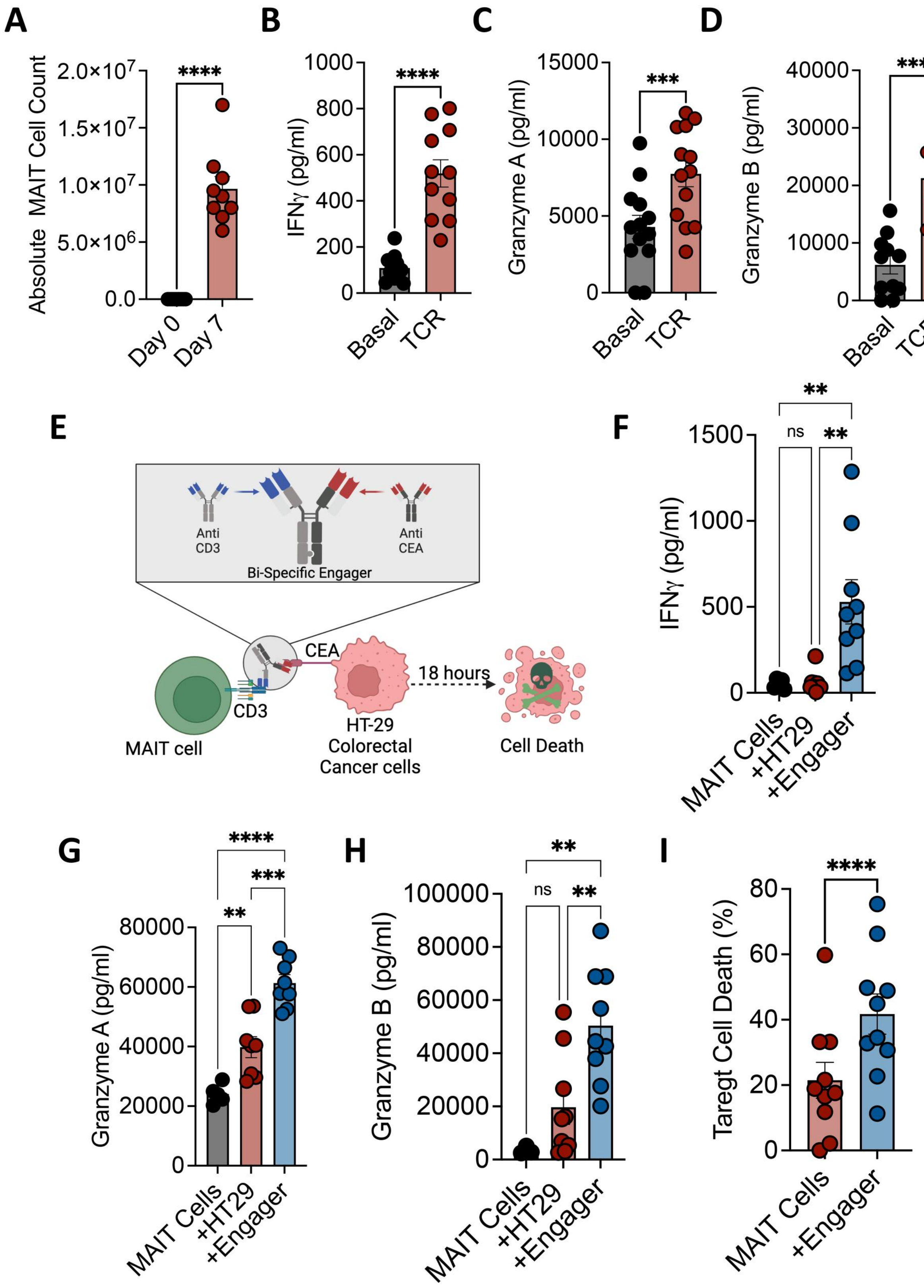

Figure 1 MAIT cells exhibit rapid proliferation upon activation and subsequently induce lysis of target cells alongside the production of cytokines. (A) Scatterplots demonstrating the rapid expansion of MAIT cells after 7 days of stimulation with 5-ARU-MG and IL-2. (B–D) Scatterplots depicting the levels of IFNγ, granzyme A, and granzyme B secretion in IL-2–expanded MAIT cells that have been stimulated with anti-CD3/CD28 TCR beads for a duration of 18 h, as quantified by ELISA. (E) A diagram of the study design, which employs the bispecific T cell engager cibistamab to direct MAIT cell cytotoxicity towards HT29 cancer cells. (F–H) A scatterplot illustrating the influence of cibistamab on the secretion levels of IFNγ, granzyme A, and granzyme B in IL-2–expanded MAIT cells that have been cocultured with HT29 cancer cells for 18 h, as determined by ELISA. (I) A scatterplot illustrating cibistamab's impact on IL-2–expanded MAIT cells' cytotoxic capability against HT29 cancer cells after an 18-h incubation period. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. CEA, carcinoembryonic antigen; ns, not significant.

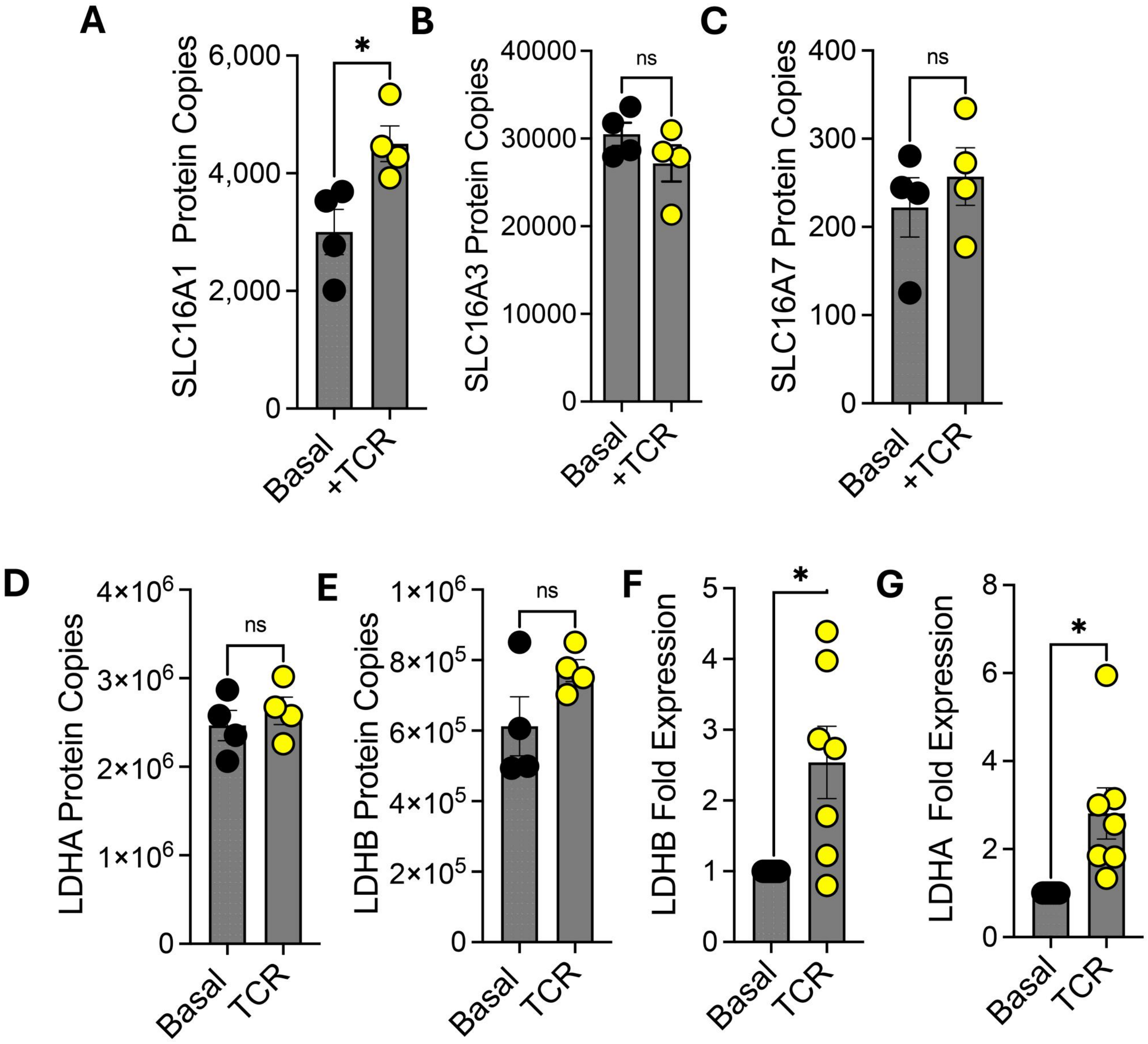

Figure 2 MAIT cells possess the necessary machinery for the transport and metabolism of lactate. (A–C) A scatterplot depicting the protein copy numbers of MCTS-SLC16A1, SLC16A3, and SLC16A7 in IL-2–expanded MAIT cells, either at a basal state or stimulated for 18 h with anti-CD3/CD28 TCR beads. (D, E) Scatterplots illustrating the protein copy numbers of LDHA and LDHB in IL-2–expanded MAIT cells, similarly analyzed at a basal state or stimulated for 18 h with anti-CD3/CD28 TCR beads. (F, G) Scatterplots showing the messenger RNA levels of *LDHA* and *LDHB* from IL-2–expanded MAIT cells that were stimulated with TCR beads for 6 h. $^*P < 0.05$. ns, not significant.

influenced MAIT cell metabolism. Based on the literature[28,29] and our own dose curve analysis (Fig. S1), 45 nM of sodium lactate was used in all subsequent experiments. Here, we performed extracellular flux analysis on MAIT cells in the presence or absence of sodium lactate upon TCR stimulation. Interestingly, our analysis revealed increased rates of oxidative phosphorylation, while glycolysis rates remained unaffected (Fig. 3A–C; Fig. S2). Notably, upon testing the mitochondrial capacity of MAIT cells treated with lactate using FCCP treatment, we observed a significant rise in mitochondrial capacity and ATP-derived from oxidative phosphorylation (Fig. 3D–F). Furthermore, we corroborated our Seahorse analysis by investigating how sodium lactate affected intracellular ATP levels in MAIT cells. Here, MAIT cells stimulated in the presence of sodium lactate had elevated levels of intracellular ATP compared to the control (Fig. 3G).

Exogenous sodium lactate boosts MAIT cell effector function and bispecific engager-mediated cytotoxicity

Next, we investigated the impact of exogenous sodium lactate in the culture system on MAIT cell effector functions. We demonstrate that sodium lactate significantly increased MAIT cell proliferation during IL-2 expansion (Fig. 4A). In parallel, we demonstrate that exogenous sodium lactate led to elevated production of the IFNγ, TNF, and the cytolytic molecules granzyme

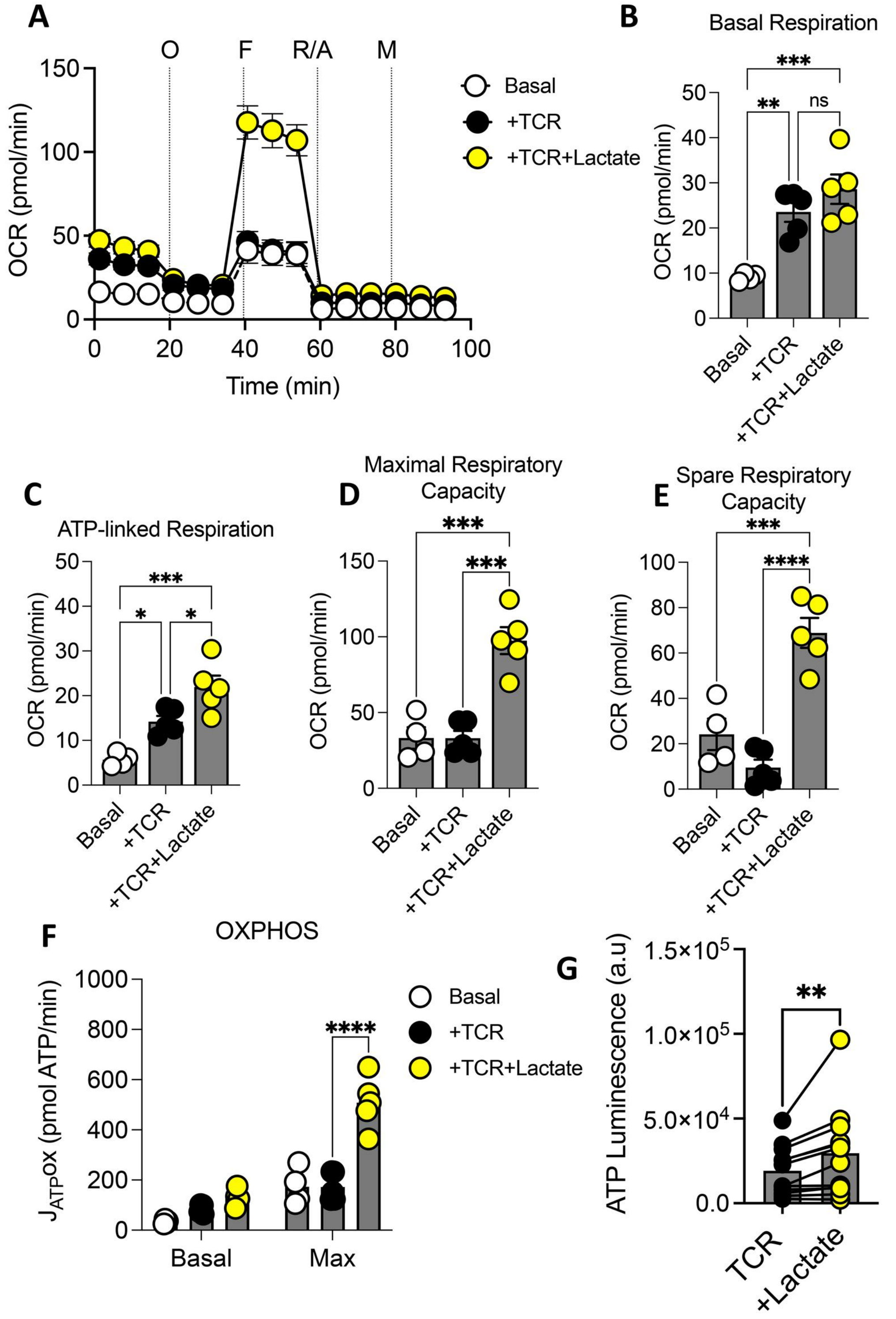

Figure 3 Exogenous sodium lactate alters the metabolism of MAIT cells. (A) Representative Seahorse trace illustrating oxygen consumption rate (OCR) in MAIT cells activated with anti-CD3/CD28 TCR beads for a duration of 24 h, in both the presence and absence of sodium lactate. (B-E) Scatterplots showing: basal respiration, ATP-linked respiration, maximal respiratory capacity, or spare respiratory capacity in MAIT cells activated with anti-CD3/CD28 TCR beads for a duration of 24 h, both in the presence and absence of sodium lactate. (F) Plot demonstrating the basal and maximal ATP production (J_{ATP}^{OX}) from oxidative phosphorylation (OXPHOS) in the presence or absence of sodium lactate. (G) Plot representing the influence of sodium lactate on ATP production in MAIT cells stimulated with anti-CD3/CD28 TCR beads for 18 h. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. ns, not significant.

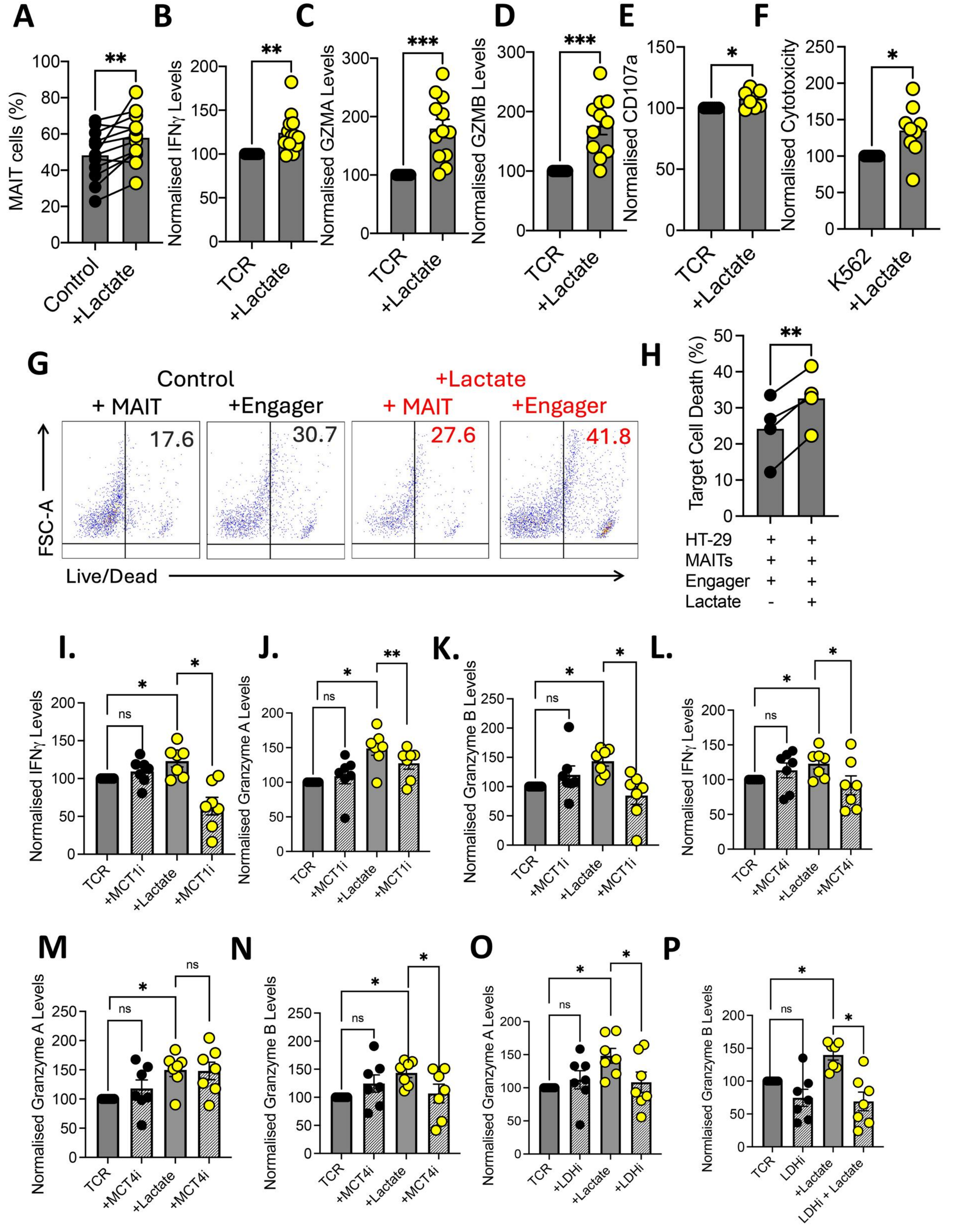

Figure 4 (A) Scatterplot displaying the effect of exogenous sodium lactate on the expansion of MAIT cells after 7 d of stimulation with 5-ARU-MG and IL-2, comparing conditions with and without sodium lactate treatment, as quantified through flow cytometry. (B–D) Scatterplots depicting the normalized quantification of IFNγ, granzyme A, and granzyme B from IL-2–expanded MAIT cell. (E) Scatterplot displaying CD107a expression on peripheral blood MAIT cells stimulated using anti-CD3/CD28 Dynabeads for 18 h in the presence of absence of sodium lactate. (F) A scatter plot showing the lysis of K562 after 3 h coculture with IL-2–expanded MAIT cells cultured in the presence of absence of sodium lactate. (G, H) Representative flow cytometry dot plot and scatterplot showing the lysis of HT29 cancer cells after an 18-h coculture with IL-2–expanded MAIT cells, with or without the bispecific T cell engager cibistamab in the presence of absence of sodium lactate. (I–P) Scatterplots depicting the normalized quantification of IFNγ, granzyme A, or granzyme B from IL-2–expanded MAIT cell stimulated using anti-CD3/CD28 Dynabeads for 18 h in the presence of absence of sodium lactate with or without the addition of (I–K) the MCT1 inhibitor AZD3965, (L–N) the MCT4 inhibitor VB124, or (O–P) the lactate dehydrogenase inhibitor (LDHi) GSK2837808A. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. FSC-A, forward scatter area; ns, not significant.

A and granzyme B but not IL-17 by TCR-stimulated MAIT cells. (Fig. 4B–D; Fig. S3). We also observed a modest increase in the expression of the degranulation marker, CD107a (Fig. 4E). Strikingly, these effects resulted in enhanced MAIT cell cytotoxicity against the K562 cancer cell line preloaded with 5-ARU-MG (Fig. 4F). To translate back to the potential use as an adoptive cell therapy with the bispecific engager, we again cocultured MAIT cells with HT29 cancer cells in the presence of a bispecific engager and found that adding exogenous sodium lactate enhanced the direct cytotoxic effects (Fig. 4G, H). Finally, we undertook a series of inhibitor studies targeting key lactate transporters (MCT1 and MCT4) and LDHA to investigate if these were required for the effect of exogenous sodium lactate on MAIT cell functional responses. For both the MCT1 and MCT inhibitors, we observed no significant effect on MAIT cell functional responses after TCR stimulation; however, we did see inhibition of MAIT cell functional responses in the presence of exogenous sodium lactate (Fig. 4I–N). Similarly, we also observed a significant impact on granzyme production with the addition of a lactate dehydrogenase inhibitor (Fig. 4O, P) supporting the concept that MAIT cells need to actively transport and metabolism the exogenous lactate to boost their function. These data collectively demonstrate that metabolic rewiring of MAIT cells boost their anticancer functions, which can be directed using bispecific T cell engagers.

Discussion

MAIT cells are potent cytotoxic immune cells capable of rapidly eliminating infected or transformed cells. Our work and others have shown that human MAIT cells can effectively target and kill various targe cell lines, such as A549 and K562.[11,31] Their robust effector functions are underpinned by the production of cytotoxic cytokines like IFNγ and cytolytic molecules such as granzyme B.[10] Notably, in preclinical murine models, MAIT cells have demonstrated the capacity to kill cancer cells and assist in tumor clearance.[14,32] As a result of these traits, MAIT cells are gaining recognition for their role in antitumor immunity. The current study focuses on novel strategies for using MAIT cells as an adoptive cellular therapy for cancer. We highlight their effectiveness for in vitro expansion, robust and targeted cytotoxicity, and capacity for metabolic reprogramming—key aspects that could be harnessed for therapeutic applications.

MAIT cells, unlike conventional T cells, do not cause graft-versus-host disease, due to their restriction by the conserved antigen presentation molecule MR1.[16,33] This further strengthens their potential as an adoptive cell therapy. This would require the ability to easily expand MAIT cells in vitro. Here, we show that large numbers of MAIT cells can be quickly amplified from human peripheral blood. Importantly, we confirm that these amplified MAIT cells are robust producers of key effector molecules including IFNγ and granzymes A and B which are critical for anticancer responses.[34–37] Excitingly, our study has demonstrated that bispecific T cell engagers can effectively redirect MAIT cells toward tumors, further amplifying their anticancer activity. While MAIT cells possess strong intrinsic cytotoxic abilities, directing them selectively to tumor cells in vivo remains a challenge encountered in cellular immunotherapy. To address this, we utilized cibistimab, a bispecific T-cell engager simultaneously targeting carcinoembryonic antigen and CD3.[38] This dual-targeting approach greatly enhanced MAIT cell activation, leading to increased production of IFNγ, granzyme B, and granzyme A, and significantly improved the direct killing of HT29 colorectal cancer cells.

Our previous studies highlighted that MAIT cells primarily depend on glycogen metabolism during the initial 6 h following activation, whereas glucose metabolism becomes crucial for responses extending up to 18 h.[11] However, this dependence on glucose may present a challenge as tumor cells engage in aerobic glycolysis, consuming large amounts of the environmental glucose and producing high lactate concentrations as a by-product.[39] This metabolic competition creates a hostile environment in which immune cells struggle to sustain their anticancer functions.[3]

Lactate has traditionally been viewed as an immunosuppressive metabolite in the TME, primarily due to its association with acidity and hypoxia—factors that are known to suppress immune responses in the TME.[40,41] However, studies in conventional T cells have demonstrated that lactate can be fuel for the cell and actually metabolically rewires the cell.[26,28,29] To see if MAIT cells can also utilize lactate as a fuel, we first investigated if they expressed the transporters and enzyme required for lactate metabolism and found that MAIT cells express transporters of the monocarboxylate family in addition to lactate dehydrogenase enzymes that facilitate the uptake and metabolism of lactate, enabling them to potentially utilize it as a carbon source for oxidative phosphorylation.

The capacity for human MAIT cells to activate alternative metabolic pathways has not been explored to date, and we show that conditioning with sodium lactate induces a shift in energy production from glycolysis to oxidative phosphorylation, resulting in improved mitochondrial respiration alongside increased ATP production. This mirrors findings in conventional CD8+ T cells.[29] Crucially, our study focuses on enhancing MAIT cells with sodium lactate in a healthy environment that ensures a well-buffered and pH-neutral state before their potential exposure to the immunosuppressive TME, which may facilitate beneficial metabolic adaptations. Our findings support the growing evidence that lactate serves as an active carbon source.[25,26] Lactate indeed functions as a psychological carbon fuel for various tissues, especially in muscle, and more recently in immune populations. Kaymak et al.[26] have shown that CD8+ T cells can integrate lactate into the tricarboxylic acid cycle, in fact, preferring it over glucose as a metabolic fuel for oxidative phosphorylation, and offering protection against bacterial infection. This provides insight into how nutrient availability can influence cellular metabolic phenotypes, which may be harnessed to enhance their immunotherapeutic potential.

Furthermore, adoptive T cell therapies tend to depend heavily on glycolysis, as in vitro expansion occurs under more hyperglycemic conditions than in vivo,[42] potentially restricting metabolic adaptability after transfer. To address this, Delgoffe and colleagues showed that conditioning chimeric antigen receptor T cells for oxidative phosphorylation enhances their longevity and effectiveness.[27,43] Interestingly, we show that introducing sodium lactate starting from day 3 of MAIT cell expansion boosts growth. By engaging in oxidative phosphorylation in vitro, MAIT cells exhibit metabolic adaptability, while further in vivo studies will be required to confirm that it is possible that this conditioning could enhance their performance in nutrient fluctuating in vivo environments, thus strengthening their therapeutic potential.

Finally, we demonstrate that supplementing expanded MAIT cells with sodium lactate significantly enhances the production of IFNγ, granzyme A, granzyme B, and TNF but not IL-17A, equipping them with an increased cytotoxic payload. The differential effect on IL-17A was not explored in the current study, but Riffelmacher et al.[24] demonstrated differential metabolic regulation of MAIT1 and MAIT17 subsets, which may explain our observation, but further work is required. Excitingly, we show that lactate conditioning also improves the direct killing of target cells. Highlighting the possible translational potential, Feng et al.[28] reported that CD8+ T cells pretreated with lactate in vitro effectively inhibited tumor growth when transferred into tumor-bearing mice. Importantly, we demonstrate that blocking the transport of lactate and the key enzyme lactate dehydrogenase using specific inhibitors negates the effect of exogenous lactate treatment on MAIT cell responses, which when paired with our metabolic reprogramming supports the concept that MAIT cells are likely utilizing the exogenous lactate as a fuel.

Current clinical trials are investigating bispecific T cell engagers like cibistimab, which effectively direct T cells to tumors.[2,38] Our findings highlight that CD3-targeting engagers can robustly activate MAIT cells, and this is boosted with lactate conditioning. Bispecific engagers are currently being developed to selectively target the MAIT cell receptor Vα7.2 while bypassing CD3, thereby preventing the activation of immunosuppressive regulatory T cells. This strategy may be particularly advantageous for solid tumor treatment, enhancing antitumor immunity while minimizing regulatory T cell–mediated immunosuppression.

Our study has some limitations, with the entirety of the work being done using in vitro models, and with a heterogenous population of human MAIT cells that display a predominant Th1 phenotype, but it builds a strong rationale for the future testing of metabolically conditioned MAIT cells paired with bispecific T cell engagers in animal models of cancer.

In conclusion, our study highlights the promising potential of MAIT cells as an adoptive cancer immunotherapy, by showing that MAIT cells are readily amplified in vitro, and these cells possess strong cytotoxic capabilities, effectively targeting and eliminating cancer cells through the production of key cytokines and cytolytic molecules. We also provide evidence that human MAIT cells can be metabolically reprogrammed with the addition of sodium lactate leading and redirected using bispecific T cell engagers, building a strong rationale for further studies in in vivo models.

Author contributions

A.B., B.J.J., N.K.M., E.K.R., and S.M. performed the experiments, carried out analysis and approved the final manuscript as submitted. O.R., C.D., H.H., and D.O.S. recruited study participants and helped with study design, analysis and approved the final manuscript as submitted. D.O.S., N.J., and A.E.H. conceptualized and designed the study, analyzed the data, drafted the manuscript, and approved the final manuscript as submitted.

A.B. (Conceptualization [Equal], Data curation [Equal], Formal analysis [Equal], Investigation [Lead], Methodology [Lead], Writing—original draft [Lead], Writing—review & editing [Equal]), B.J.J. (Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Methodology [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), N.K.-M. (Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), E.K.R. (Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), S.M. (Investigation [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), O.R. (Data curation [Equal], Resources [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), C.D. (Data curation [Equal], Investigation [Equal], Resources [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), H.H. (Conceptualization [Equal], Project administration [Equal], Resources [Equal], Supervision [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), D.O. (Conceptualization [Equal], Funding acquisition [Equal], Resources [Equal], Supervision [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), N.J. (Conceptualization [Equal], Data curation [Equal], Funding acquisition [Equal], Investigation [Equal], Project administration [Equal], Supervision [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), and A.H. (Conceptualization [Equal], Funding acquisition [Lead], Investigation [Lead], Project administration [Lead], Supervision [Lead], Writing—original draft [Lead], Writing—review & editing [Lead])

Supplementary material

Supplementary material is available at *The Journal of Immunology* online.

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Conflicts of interest

The authors declare no conflict of interest.

Data availability

All data related to this study are included in the manuscript and the supplementary materials.

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