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Article *in* The Journal of Immunology · May 2019

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J Immunol 2019; 202:3404-3411; Prepublished online 10 May 2019;
doi: 10.4049/jimmunol.1801600
http://www.jimmunol.org/content/202/12/3404

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Obesity Reduces mTORC1 Activity in Mucosal-Associated Invariant T Cells, Driving Defective Metabolic and Functional Responses

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Obesity underpins the development of numerous chronic diseases, such as type II diabetes mellitus. It is well established that obesity negatively alters immune cell frequencies and functions. Mucosal-associated invariant T (MAIT) cells are a population of innate T cells, which we have previously reported are dysregulated in obesity, with altered circulating and adipose tissue frequencies and a reduction in their IFN-γ production, which is a critical effector function of MAIT cells in host defense. Hence, there is increased urgency to characterize the key molecular mechanisms that drive MAIT cell effector functions and to identify those which are impaired in the obesity setting. In this study, we found that MAIT cells significantly upregulate their rates of glycolysis upon activation in an mTORC1-dependent manner, and this is essential for MAIT cell IFN-γ production. Furthermore, we show that mTORC1 activation is dependent on amino acid transport via SLC7A5. In obese patients, using RNA sequencing, Seahorse analysis, and a series of in vitro experiments, we demonstrate that MAIT cells isolated from obese adults display defective glycolytic metabolism, mTORC1 signaling, and SLC7A5 aa transport. Collectively, our data detail the intrinsic metabolic pathways controlling MAIT cell cytokine production and highlight mTORC1 as an important metabolic regulator that is impaired in obesity, leading to altered MAIT cell responses. *The Journal of Immunology*, **2019, 202: 3404–3411.**

Obesity is a global epidemic, impacting over 600 million adults and 150 million children (1). Obesity is linked to numerous chronic diseases, including type II diabetes, cardiovascular disease, and certain cancers (2). Underpinning the obesity-driven development of chronic disease is systemic inflammation and immune cell dysregulation (3, 4). We have previously reported the negative impact of obesity on several immune cell populations, including invariant NK T cells, NK cells, dendritic cells, and mucosal-associated invariant T (MAIT) cells (5–8).

MAIT cells are a population of non-MHC–restricted T cells that are important in the immune defense against bacterial and viral infections. MAIT cells are early-responding T cells that are capable of rapidly producing multiple cytokines upon activation, such as IFN-γ, TNF-α, and IL-17 (9). MAIT cells are activated when their invariant TCR recognize bacterial derivatives presented on the MHC-like molecule MR1 (9, 10). MAIT cells can also be activated in a TCR-independent manner via cytokine stimulation (11). Dysregulated MAIT cell cytokine profiles have been reported in several diseases, including obesity, arthritis, and viral

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Received for publication December 7, 2018. Accepted for publication April 12, 2019.

This work was supported by the National Children's Research Centre. A.E.H. and D.O. are funded by the Health Research Board (HRB) (Grant RA-POR-2014-593), and A.O. is funded by the HRB Centre for Health and Diet Research (Grant HRC2014/13), both of which are funded by the Irish HRB. M.M.P., N.A.W.W., and A.E.H. are supported by the National Children's Research Centre. The Seahorse XF-96 Analyzer is funded by Science Foundation Ireland Grant 16/RI/3399.

A.O., R.M.L., M.M.P., L.M.T., N.A.W.W., F.C.T., R.B., and C.B. performed the experiments and carried out analysis and approved the final manuscript as submitted. A.M. recruited patients and controls. N.V. and G.S.B. provided MAIT cell reagents and aided in the design of MAIT cell activation experiments. G.S. and C.F. performed and analyzed the RNA sequencing. A.E.H., L.V.S., P.N.M., D.K.F., R.M.L., L.L., and D.O. conceptualized and designed the study, analyzed the data, drafted the manuscript, and approved the final manuscript as submitted.

The sequences presented in this article have been submitted to the Gene Expression Omnibus under accession number GSE126169.

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The online version of this article contains supplemental material.

Abbreviations used in this article: BCH, 2-aminobicyclo- (2, 2, 1)-heptane-2-carboxylic acid; 2DG, 2-deoxyglucose; ECAR, extracellular acidification rate; FMO, flow minus-1; MAIT, mucosal-associated invariant T; OxPhos, oxidative phosphorylation; ps6, ribosomal S6 protein; RNA-Seq, RNA sequencing.

infection (8, 12–15). The pathways controlling MAIT cell cytokine responses are still under investigation.

It is now well established that cellular metabolism plays a critical role in determining immune cell responses (16). Extrinsic and intrinsic signals result in immune cell metabolic reprogramming, which provides both the energy and biological intermediates required for a robust immune response. Although different lymphocyte subsets can adopt distinct metabolic signatures, in general, effector lymphocytes are characterized by elevated glucose metabolism with high rates of glycolysis and oxidative phosphorylation (OxPhos) (16).

Currently, there is a paucity of data regarding the metabolic profile of MAIT cells or the importance of cellular metabolism in driving MAIT cell effector functions. In this study, we show that MAIT cells use glycolytic metabolism upon activation. Importantly, we show the requirement of this elevated rate of MAIT cell glycolysis for cytokine production. Using RNA sequencing (RNA-Seq) and Seahorse analysis, we show impaired glycolytic metabolism in MAIT cells from obese individuals. We show that MAIT cell glycolysis is dependent on mTORC1 activation, and in obesity mTORC1 activation is reduced. Finally, we show that mTORC1 activation in MAIT cells is dependent on amino acid transport via SLC7A5. Collectively, our data show for the first time, to our knowledge, the intrinsic metabolic pathways controlling MAIT cell cytokine production. Furthermore, we implicate a novel amino acid–dependent regulatory mechanism that is concurrent with impaired MAIT cell functions in obesity.

Materials and Methods

Study cohorts

A cohort of 30 obese adults (mean body mass index 45.1) was enrolled into this study, along with an age- and sex-matched cohort of healthy lean controls (mean body mass index 24.7) (Supplemental Table I). Exclusion criteria included patients with comorbid inflammatory conditions outside of type II diabetes. Patients who smoke or are on anti-inflammatory medications, GLP-1 therapies, or sodium-glucose cotransporter 2 inhibitors were also excluded.

Preparation of PBMCs and flow cytometric analysis

PBMC samples were isolated by density centrifugation from fresh venous blood samples. MAIT cell staining (1×10^6 PBMC) was performed using specific surface mAbs (all Miltenyi Biotec), namely, CD3, CD161, CD8, and TCRVα7.2 (Supplemental Fig. 3). Cell populations were acquired using a BD FACSCanto II and analyzed using FlowJo software (Tree Star). Results are expressed as a percentage of the parent population, as indicated, and determined using flow minus-1 (FMO) and unstained controls.

MAIT cell RNA-Seq and analysis

Highly pure MAIT cells were isolated from peripheral blood samples using FACS (MoFlo XDP Sorter). RNA was extracted from cells using the RNeasy Mini Isolation Kit (Qiagen) and analyzed for quality control and quantification purposes using the Agilent Bioanalyzer and RNA Pico assay (Agilent Technologies). RNA-Seq libraries were prepared using the SMART-Seq v4 Ultra Low Input RNA Kit (Clontech Laboratories), and next-generation sequencing was carried out using Illumina NextSeq 500 Single-End 75 bp (SE75) chemistry. Single-end RNA-Seq reads were aligned to the human hg38 reference genome using Tophat2 (17) and Bowtie (18) and the Ensembl gene annotation downloaded from the BioMart Web site. The Qlucore Omics Explorer 3.2 software package was used for visualization of RNA-Seq data. Qlucore Omics software converted imported bam files into read counts and then normalized these across the samples using the trimmed mean of M-values method (19). Principal component analysis was performed using the default setting: mean = 0, variance = 1 normalization based on 503 genes that passed filtering by variance set at the level of 0.25. Gene set enrichment analysis ranked genes based on the correlation between their expression, and the normalized enrichment score reflected the degree to which Hallmark gene sets from the Molecular Signatures Database were overrepresented from the ranked gene list (20). Differential gene expression analysis was implemented in the Qlucore, which performed a two-group comparison between lean and obese MAIT cells. Genes downregulated in obesity within the glycolysis and MTORC1 gene sets with a p value <0.05 and $\log_2$ fold change >1.5 were represented in the heat maps generated in Qlucore. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE126169.

MAIT cell cytokine production analysis

MAIT cell cytokine production was determined by intracellular flow cytometry. Briefly, PBMC (1×10^6) were cultured in the presence of a protein transport inhibitor in media alone (control) or stimulated with either TCR microbeads (Miltenyi Biotec), IL-12, IL-18, IL-12/18 (all BioLegend), or a combination of TCR microbeads and IL-12/IL-18 for 18 h at 37°C. Additionally, metabolic inhibitors were added as indicated: rapamycin (20 nM), 2-deoxyglucose (2DG) (10 μM), oligomycin (2 μM), or 2-aminobicyclo- (2, 2, 1)-heptane-2-carboxylic acid (BCH) (50 mM). For acute glucose restriction experiments, PBMC were stimulated in media containing less than 1 mM glucose for 18 h, after which cells were investigated for intracellular cytokine production by flow cytometry, as previously described.

MAIT cell cytokine production by ELISpot

MAIT cell cytokine production was also determined by ELISpot. Briefly, 96-well ELISpot plate(s) were precoated with mAbs to IFN-γ overnight at 4°C. Plates were washed in PBS to remove coating Ab and incubated at 37°C for 1 h in complete RPMI. Previously isolated MAIT cells (2×10^5) were cultured on the precoated plates either basally or stimulated with IL-12/IL-18 and TCR microbeads at 37°C for 18 h. ELISpot plates were then washed with PBS/Tween for a minimum of six washes. Following this, 50 μl of biotinylated mouse anti-human IFN-γ was added and incubated at room temperature for 3 h. ELISpot plates were washed in wash buffer, and antibiotin-labeled Ab was added to the wells and incubated at room temperature for 2 h. Finally, prefiltered BCIP/NBT solution (50 μl) was added to each well and incubated at room temperature for 5 min or until distinct dark spots appeared in the positive-control wells. Plates were rinsed with tap water and allowed to air dry overnight. Cytokine expression was analyzed using a semiautomated ELISpot plate reader.

MAIT cell glucose consumption analysis

Fresh PBMC (1×10^6 ml) were activated as previously described for 18 h; cells were then washed and transferred to glucose-free media in the presence of the fluorescent glucose analogue 2NBDG (Life Technologies) for 2 h. Additionally, glut 1 expression was examined on basal and activated MAIT cells using an mAb specific for Glut-1 (R&D Systems). FMO and unstimulated PBMC were used as negative controls.

MAIT cell Seahorse analysis

For real-time analysis of the extracellular acidification rate (ECAR) and oxygen consumption rate of purified MAIT cells cultured under various conditions, a Seahorse XF-96 Analyzer (Seahorse Bioscience) was used. In brief, 200,000 purified, expanded MAIT cells were adhered to a CellTaq (BD Pharmingen)–coated 96-well XF Cell Culture Microplate (Seahorse Bioscience). Sequential measurements of ECAR and oxygen consumption rate following addition of the inhibitors (Sigma-Aldrich) oligomycin (2 μM), FCCP (0.5 μM), rotenone (100 nM) plus antimycin A (4 μM), and 2DG, (30 mM) allowed for the calculation of basal glycolysis, glycolytic capacity, basal OxPhos, and maximal mitochondrial capacity. Each cell culture condition was evaluated in quadruplicate, and 14 measurements were made per sample.

MAIT cell mTOR analysis

PBMC (1×10^6 ml) were activated as previously described for 18 h. mTOR activity in MAIT cells and $CD8^+$ T cells was examined via the phosphorylation of ribosomal S6 protein (pS6) (Cell Signaling Technology) by flow cytometry. Rapamycin treatment was used as a control for pS6 specificity and to determine the requirement of mTORC1 for IFN-γ production. FMO and unstimulated PBMC cells were used as negative controls.

MAIT cell amino acid uptake and BCH experiments

PBMC or MAIT cells were used to investigate kynurenine uptake as described in (21). Briefly, after surface Ab staining of samples, cells were resuspended in 200 ml of warmed HBSS (1×10^6 cells) and incubated in a water bath at 37°C. Kynurenine (200 mM in HBSS), BCH (20 mM in HBSS), and HBSS were warmed to 37°C and added as appropriate. Uptake was stopped after 4 min by PFA (final concentration 1%) for 30 min at room temperature in the dark. After fixation, cells were washed twice in

PBS/0.5% BSA and resuspend in PBS/0.5% BSA prior to acquisition on flow cytometer. The 405-nm laser and 450/50 BP filter were used for kynurenine fluorescence detection. For SLC7A5 inhibition experiments, the concentration of amino acids in RPMI 1640 was diluted two-fold using HBSS (Invitrogen) in the presence or absence of BCH (50 mM; Sigma-Aldrich).

Statistics

Statistical analysis was completed using GraphPad Prism 6 Software. Data are expressed as SEM. We determined differences between two groups using Student *t* test and Mann–Whitney *U* test where appropriate. Analysis across three or more groups was performed using ANOVA. Correlations were determined using linear regression models and expressed using Pearson or Spearman rank correlation coefficient, as appropriate. The *p* values were expressed with significance set at <0.05.

Study approval

Ethical approval was granted by the Medical Research Ethics Committees at St. Vincent's University Hospital. All patients gave written informed consent prior to partaking in the study.

Results

Increased rates of glycolysis are essential for driving MAIT cell IFN-γ production

MAIT cells are potent producers of cytokine (IFN-γ and TNF-α among the most abundant). We show that in a stimulation-dependent manner, a large proportion of MAIT cells produce IFN-γ and TNF-α but not IL-17, with the most robust response seen after stimulation with anti-CD3/CD28–coated beads (TCR beads) and the cytokines IL-12/IL-18 (Fig. 1A, Supplemental Figs. 1A, 3). The metabolic pathways used by MAIT cells during their cytokine production are currently undefined. We show that MAIT cells express the major glucose transporter Glut-1 and upregulate the uptake of the fluorescent 2DG analogue 2NBDG upon activation in a stimulus-dependent manner (Fig. 1B–D). These data suggested that MAIT cells upregulate glucose metabolism. Therefore, we next performed detailed metabolic analysis using the Seahorse extracellular flux analyzer. Interestingly, we found MAIT cells specifically upregulate their rates of glycolysis (Fig. 1E, 1F), but not OxPhos (Supplemental Fig. 2B), upon activation. In addition, the glycolytic capacity, ECAR following oligomycin injection, of MAIT cells also increased significantly upon stimulation, which suggests that these cells are increasing the expression of the glycolytic machinery (Fig. 1E). Therefore, overall there was a pronounced shift toward glycolytic metabolism in activated MAIT cells. To assess if cellular glycolysis was required for cytokine production by MAIT cells, we used low doses of the specific glycolytic inhibitor 2DG. The data showed that MAIT cell IFN-γ production was significantly reduced when the rate of glycolysis was limited with 2DG (Fig. 1G, 1H). Interestingly, 2DG treatment had no impact on TNF-α production by MAIT cells (Fig. 1I). In contrast, inhibition of mitochondrial respiration with the ATP synthase inhibitor oligomycin did not reduce IFN-γ production by MAIT cells (Supplemental Fig. 2D, 2E) but did reduce TNF-α (Supplemental Fig. 2F). To further test the requirement for glucose metabolism in driving MAIT cell cytokine responses, we cultured MAIT cells under acute glucose restriction and showed a strong inhibition of IFN-γ, further

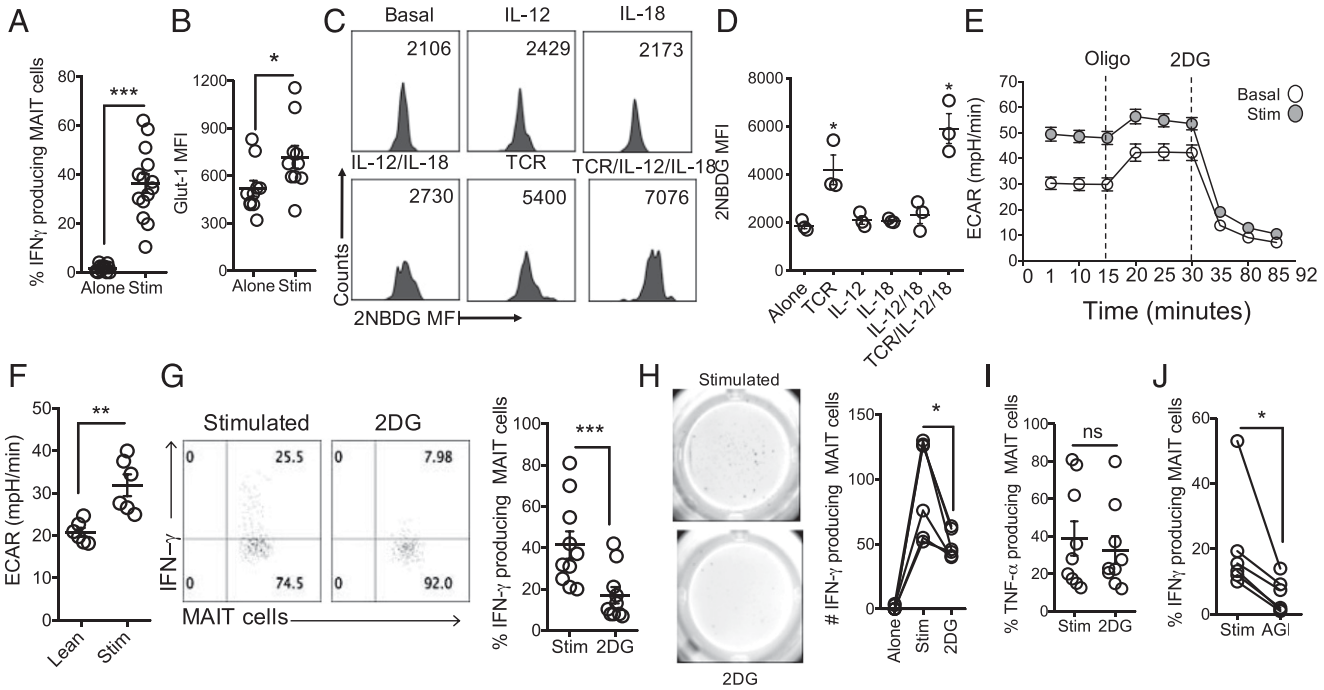

FIGURE 1. MAIT cells use glycolytic metabolism during IFN-γ production. (**A**) Scatter plot showing MAIT cell production of IFN-γ in a cohort of lean adults (unstimulated versus stimulated with TCR microbead/IL-12/IL-18). (**B**) Scatter plot showing Glut-1 expression (MFI) on either basal or stimulated MAIT cells from lean adults ($n = 10$). (**C** and **D**) Representative histograms and scatter plot showing 2NBDG uptake by MAIT cells stimulated with either TCR microbeads, IL-12, IL-18, both IL-12 and IL-18, or TCR microbeads and IL-12/IL-18 ($n = 3$). (**E** and **F**) Scatter plot and representative Seahorse traces displaying MAIT cells ECAR rates in either basal (open circles) or stimulated (gray circles) MAIT cells from lean adults. Data representative of six independent experiments. (**G**) Representative flow cytometry dot plots and scatter plot displaying the effect of 2DG treatment on IFN-γ production by stimulated MAIT cells ($n = 10$). (**H**) Representative ELISpot wells and scatter plot displaying the effect of 2DG treatment (bottom panel) on IFN-γ production by stimulated MAIT cells ($n = 5$). (**I**) Scatter plot displaying the effect of 2DG treatment on TNF-α production by stimulated MAIT cells ($n = 10$). (**J**) Scatter plot showing impact of acute glucose restriction (AGR) on IFN-γ production by stimulated MAIT cells from lean adults ($n = 5$). Data representative of five independent experiments. Statistical comparisons using Student *t* test or ANOVA. Data representative of a minimum of 10 independent experiments unless otherwise stated. Error bars represent SEM. $*p < 0.05$, $**p \leq 0.01$, $***p < 0.001$. MFI, mean fluorescence intensity.

supporting our findings that MAIT cell IFN-γ production is dependent on glycolytic metabolism (Fig. 1J).

Defective IFN-γ production by MAIT cells in obesity parallels defective cellular metabolism

We have previously reported defective MAIT cells in obese individuals, including impaired IFN-γ production, which we confirm in an expanded cohort of obese adults (Fig. 2A). Interestingly, in our obese cohort, we did not observe defective $CD8^+$ T cell IFN-γ production (Fig. 2B). To investigate the mechanism(s) driving defective IFN-γ production by MAIT cells in obesity, we performed RNA-Seq of MAIT cells isolated from five lean and four obese individuals. Normal enrichment score analysis highlighted a reduction in the glycolytic metabolism pathway (Fig. 2C). To investigate the significance of the reduced glycolytic metabolism in obesity, Glut-1 expression and 2NBDG uptake assays were performed. The data showed that MAIT cells from obese adults show no significant difference in Glut-1 expression (Fig. 2D). However, there was a significant decrease in the uptake of 2NBDG in stimulated MAIT cells from obese individuals when compared with healthy adults (Fig. 2E). Detailed metabolic analysis was used to confirm the alterations in MAIT cell glycolytic metabolism between healthy and obese MAIT cells. In line with the reduced glycolytic gene expression and glucose uptake, activated MAIT cells from obese adults do not increase their rate of glycolysis (Fig. 2F, 2G). Indeed, MAIT cells from obese adults show reduced glycolytic capacity when compared with lean adults (Fig. 2H). Furthermore, acute glucose restriction had no additional impact/reduction on IFN-γ production in MAIT cells from obese individuals in comparison with the significant effect observed in MAIT cells from lean individuals (Fig. 2I). These data confirm that MAIT cells from obese adults are not performing increased rates of glycolysis during their activation.

mTORC1 activity is essential for driving MAIT cell glycolysis and is reduced in obesity

The signaling complex mTORC1 acts as both a nutrient sensor and a central metabolic regulator of effector immune cells. Studies have shown that mTORC1 is intrinsically linked with glycolytic metabolism in effector lymphocytes (22, 23). Strikingly, our RNA-Seq data revealed defective mTORC1 signaling. (Fig. 3A). To investigate a role for mTORC1 in driving MAIT cell metabolism, we measured mTORC1 activity in MAIT cells upon stimulation and demonstrate a robust increase as measured via the phosphorylation of pS6 (Fig. 3B, 3C). Importantly, these elevated levels of pS6 were prevented by the mTORC1 inhibitor rapamycin, demonstrating the specificity of this assay for mTORC1 signaling (Fig. 3D). We next investigated if mTORC1 signaling is required for increased rates of glycolysis in activated MAIT cells. Indeed, MAIT cells stimulated in the presence of rapamycin had significantly lower levels of glycolysis (Fig. 3E, 3F). As expected, rapamycin treatment had no significant impact on the ECAR of MAIT cells

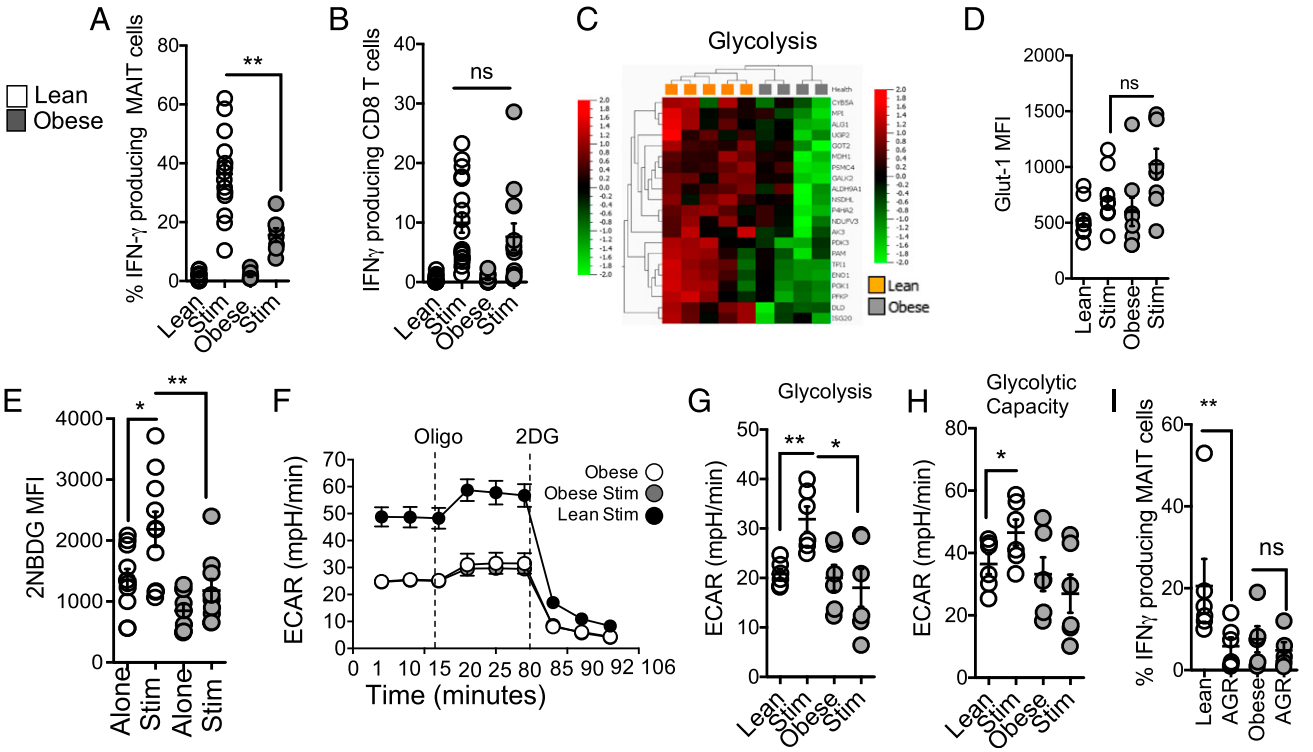

FIGURE 2. MAIT cells from obese adults display defective glycolytic metabolism. (**A**) Scatter plot displaying MAIT cell production of IFN-γ in a cohort of lean (white circles) and obese (gray circles) adult (unstimulated versus stimulated with TCR microbead/IL-12/IL-18) ($n = 15$). (**B**) Scatter plot displaying CD8 T cell production of IFN-γ in a cohort of lean (white circles) and obese (gray circles) adult (unstimulated versus stimulated with TCR microbead/IL-12/IL-18) ($n = 15$). (**C**) Heat map depicting differentially downregulated genes (p value < 0.05) within the glycolysis gene set in MAIT cells isolated from lean and obese donors ($n = 5$ and 4, respectively). (**D**) Scatter plot showing Glut-1 MFI on either basal or stimulated MAIT cells from lean (white circles) or obese (gray circles) donors ($n = 10$). (**E**) Scatter plot showing 2NBDG MFI on either basal or stimulated MAIT cells from lean (white circles) or obese (gray circles) donors. (**F**) Representative Seahorse trace displaying MAIT cell ECAR rates in either basal (open circles) or stimulated (gray circles) MAIT cells from an obese adult with representative stimulated lean donor (black circles). (**G** and **H**) Scatter plot displaying glycolysis levels (ECAR) or glycolytic capacity (ECAR post–oligomycin treatment) from lean (white circles) or obese (gray circles) donors ($n = 6$). (**I**) Scatter plot showing impact of acute glucose restriction (AGR) on IFN-γ production by stimulated MAIT cells from lean (white circles) or obese (gray circles) adults. Data representative of a minimum of five independent experiments unless otherwise stated. Statistical comparisons using Student t test or ANOVA. Error bars represent SEM. $*p < 0.05$, $**p \leq 0.01$. MFI, mean fluorescence intensity.

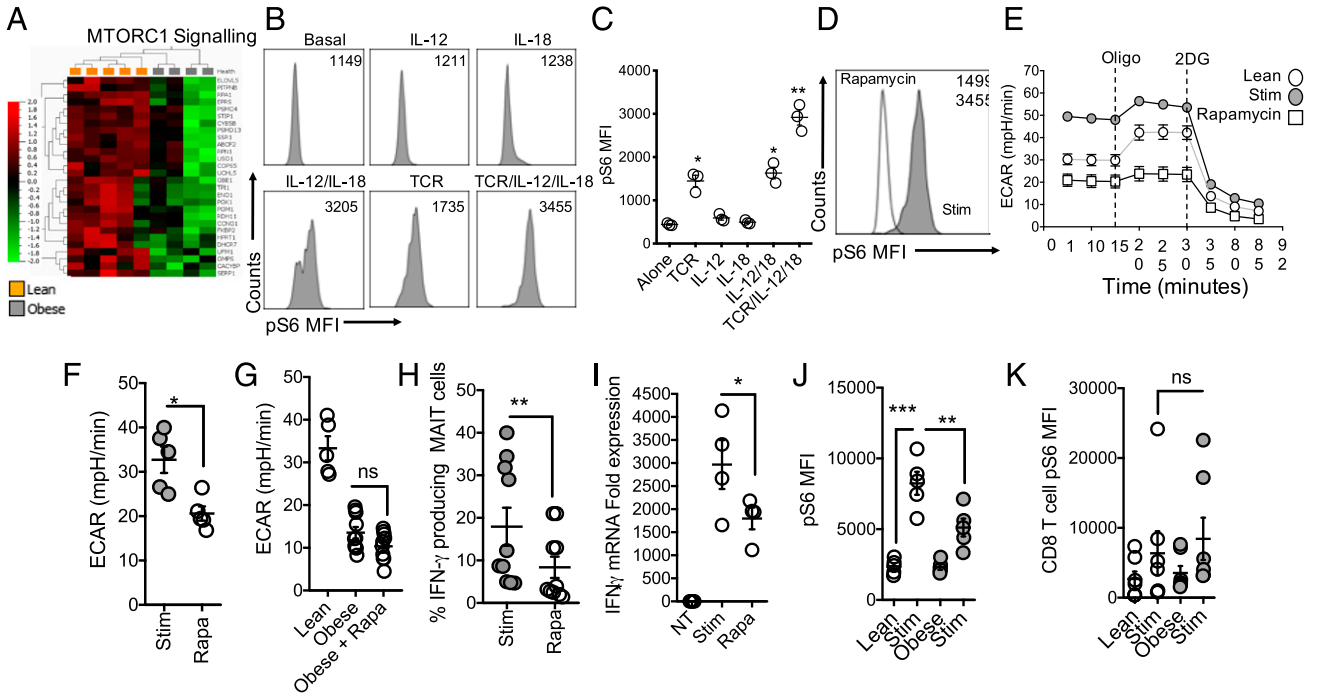

FIGURE 3. MAIT cell glycolytic metabolism is dependent on mTORC1 activation, which displays defects in obesity. (**A**) Heat map depicting differentially downregulated genes (p value < 0.05) within the mTORC1 gene set in MAIT cells isolated from lean and obese donors ($n = 5$ and 4, respectively). (**B** and **C**) Representative histogram and scatter plot showing the activation of mTORC1 (phosphorylation of S6) in MAIT cells stimulated with either TCR microbeads, IL-12, IL-18, both IL-12 and IL-18, or TCR microbeads, IL-12 and IL-18 ($n = 3$). (**D**) Representative histogram showing the inhibition of S6 phosphorylation by rapamycin in stimulated MAIT cells. (**E**) Representative Seahorse traces displaying MAIT cell ECAR rates in either basal (open circles), stimulated (gray circles), or rapamycin-treated (open squares) in MAIT cells from lean donors. (**F**) Scatter plot displaying ECAR levels in stimulated (gray circles) or rapamycin-treated (white circles) MAIT cells from lean donors. (**G**) Scatter plot detailing effect of rapamycin on ECAR rates in stimulated obese MAIT cells. (**H**) Scatter plot detailing effect of rapamycin on IFN-γ production by stimulated MAIT cells from lean donors. (**I**) Scatter plot detailing effect of rapamycin on IFN-γ mRNA in MAIT cells from lean donors. (**J**) Scatter plot showing pS6 MFI in either basal or stimulated MAIT cells from lean (white circles) or obese (gray circles) donors. (**K**) Scatter plot showing pS6 MFI in either basal or stimulated CD8 T cells from lean (white circles) or obese (gray circles) donors. Data representative of a minimum of five independent experiments unless otherwise stated. Statistical comparisons using Student t test or ANOVA. Error bars represent SEM. $*p < 0.05$, $**p \leq 0.01$, $***p < 0.001$.

from obese donors (Fig. 3G). We show that rapamycin treatment of activated MAIT cells from lean individuals inhibits IFN-γ, and this regulation occurs at an mRNA level (Fig. 3H, 3I). Together, these data demonstrate that mTORC1 is required for metabolic and functional MAIT cell responses. Indeed, the data show that activation-induced mTORC1 activity was significantly reduced in MAIT cells from obese adults (Fig. 3J). Similar to $CD8^+$ T cell IFN-γ production, we observed no difference in $CD8^+$ T cell mTORC1 activity between lean and obese donors (Fig. 3K). Collectively, these data highlight an important role for mTORC1 signaling in MAIT cell metabolism and highlight obesity-associated defects in mTORC1 as a possible driver of defective MAIT cell function.

mTORC1 activity is dependent on amino acid flux via SLC7A5, which is diminished in obesity

The data thus far highlight a crucial role for mTORC1 signaling in controlling MAIT cell metabolism and function. We next investigated the upstream regulators of mTOR activity in MAIT cells in the hopes that it may shed light on the cause behind the defective mTORC1 signaling observed in obese MAIT cells. Previous studies have highlighted the importance of amino acid transport through the system L amino acid transporter, SLC7A5, in controlling mTOR activity in primary T cells (24). To assess whether amino acid transport controls mTORC1 activity in MAIT cells, we first investigated amino acid transport in MAIT cells using a recently described flow cytometric–based assay (21). We show that MAIT cells readily uptake exogenous amino acids (as determined by active transport of the fluorescent system L transporter substrate kynurenine) (Fig. 4A, 4B). Furthermore, we show that treatment with the system L amino acid transport inhibitor BCH reduced kynurenine uptake by MAIT cells (Fig. 4C, 4D). We next investigated if MAIT cells from obese donors had altered amino acid uptake. Interestingly, we observed a significant decrease in uptake through system L transport compared with MAIT cells from lean donors (Fig. 4E, 4F). To investigate this reduction, we measured the expression of the predominant system L amino acid transporter expressed in T cells, SLC7A5, and show reduced mRNA expression in obese donors (Fig. 4G). Finally, using BCH treatment, we investigated the impact of this diminished amino acid uptake on mTORC1 activity and glycolytic metabolism in MAIT cells from healthy donors. We observed reduced mTORC1 activation and strong abrogation of glycolytic metabolism with BCH (Fig. 4H–K). As expected, we observed reduced IFN-γ production by MAIT cells treated with BCH (Fig. 4L). These data implicate for the first time, to our knowledge, a crucial role for amino acid availability in regulating MAIT cell metabolism and function in obesity.

Discussion

In this study, we show definitively that MAIT cells increase their rates of glucose uptake and glycolysis upon activation. We also show that this increased rate of MAIT cell glycolysis is essential for driving IFN-γ production. Furthermore, we identify mTORC1 as a key metabolic regulator that controls the rate of MAIT cell

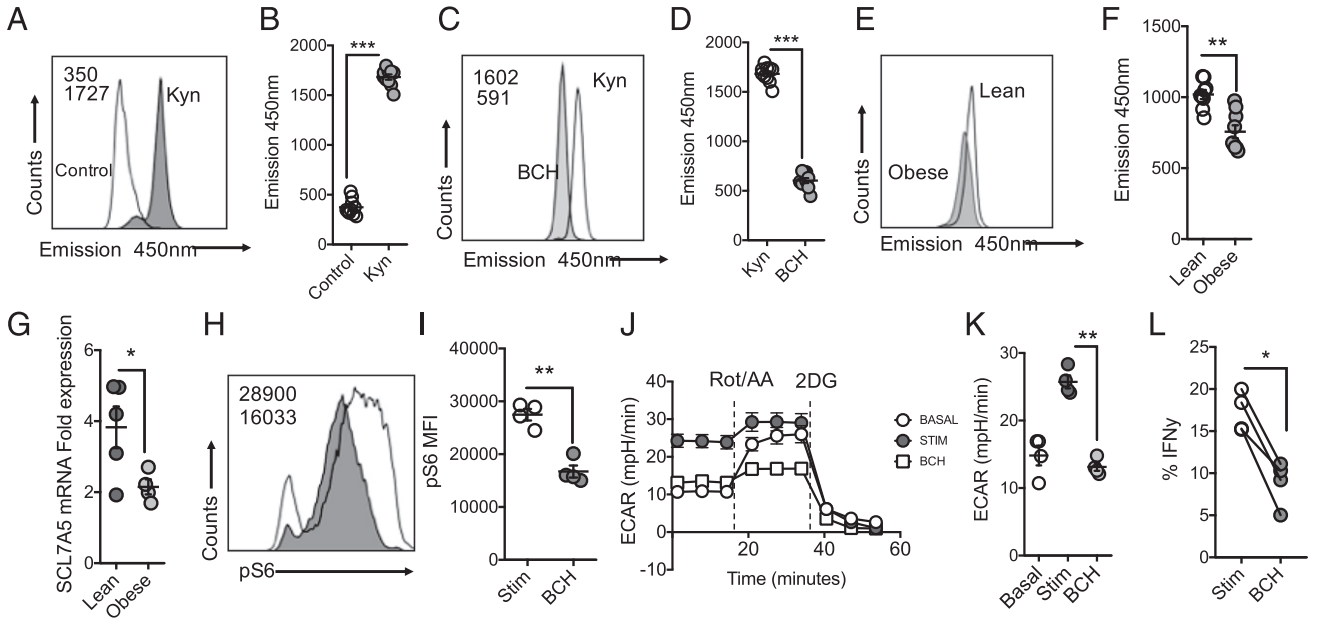

FIGURE 4. mTORC1 activation in MAIT cells is dependent on amino acid uptake via SLC7A5, which is defective in obesity. (**A** and **B**) Representative histogram and scatter plot showing Kynurenine (Kyn) uptake by MAIT cells compared with HBSS control (no Kyn) ($n = 10$). (**C** and **D**) Representative histogram and scatter plot showing the effect of BCH (50 mM) on Kyn uptake by MAIT cells ($n = 10$). (**E** and **F**) Representative histogram and scatter plot showing Kyn uptake by MAIT cells from a lean (open histogram) or obese donor (gray histogram) ($n = 10$). (**G**) Scatter plot showing SLC7A5 mRNA expression in MAIT cells from lean or obese donors ($n = 5$). (**H** and **I**) Representative histogram and scatter plot showing the effect of BCH (50 mM) on the activation of mTORC1 (pS6) in TCR-/IL-12–/IL-18–activated MAIT cells ($n = 4$). (**J** and **K**) Representative Seahorse trace and scatter plot showing the effect of BCH (50 mM) on the ECAR of TCR-/IL-12–/IL-18–activated MAIT cells ($n = 4$). (**L**) Scatter plot showing the effect of BCH (50 mM) on IFN-γ production by TCR-/IL-12–/IL-18–activated MAIT cells ($n = 4$). Data representative of a minimum of five independent experiments unless otherwise stated. Statistical comparisons using Student *t* test or ANOVA. Error bars represent SEM. $*p < 0.05$, $**p \leq 0.01$, $***p < 0.001$.

metabolism and thereby effector molecule production. We show that mTORC1 activation is under the control of amino acid uptake by the cell and expression of the system L amino acid transporter, SLC7A5. We previously reported that MAIT cells isolated from obese individuals are functionally defective. To understand the mechanisms at play, we used RNA-Seq to identify alterations in the gene expression of MAIT cells isolated from obese individuals. These analyses highlighted alterations in both glycolytic and mTORC1 pathways in MAIT cells isolated from obese individuals. Indeed, we confirm our sequencing data by showing reduced rates of glycolysis and mTORC1 activity in MAIT cells from obese adults, which parallel the loss of IFN-γ production reported in obesity (8). Finally, we provide evidence that defects in amino acid transport contribute to the described defective MAIT cell metabolism in obese patients.

MAIT cells are potent effector T cells, capable of rapidly producing multiple cytokines (9). During viral infections, activation of the MAIT cell is independent of the TCR but dependent on IL-18. Data from Slichter and colleagues (11) show that MAIT cells respond weakly to TCR stimulation in the absence of secondary stimulation by cytokine. Our data support this finding, showing that MAIT cells respond most strongly when stimulated via the TCR and cytokine receptors, specifically IL-18 and IL-12.

The pathways regulating MAIT cell cytokine production are poorly understood; however, a recent study by Zinser and colleagues (25) provided evidence that MAIT cell production of granzyme B was dependent on glycolytic metabolism. In other effector lymphocytes, it is well established that the cytokine profiles are regulated by intrinsic cellular metabolism (26–28). Activated T cells have been shown to have a large glucose requirement, especially Th 1 and 17 subsets along with $CD8^+$ cells (27). We show that MAIT cells express the glucose transporter Glut-1 and upon cytokine stimulation increase their uptake of the fluorescent 2DG analogue 2NBDG. Like the cytokine responses, MAIT cells increased 2NBDG uptake with TCR stimulation; however, the greatest increases in glucose uptake were observed with TCR–cytokine costimulation. This is in keeping with the requirement for greater amounts of energy and biological intermediates following TCR/cytokine stimulation.

Studies in effector populations such as $CD8^+$ T cells and NK cells have demonstrated that the cytokine production by these cells is dependent on glycolytic metabolism (20, 27). We investigated the metabolic pathways used by MAIT cells using the Seahorse extracellular flux analyzer and show that MAIT cells are glycolytic cells, increasing their rates of glycolysis upon activation. Interestingly, in contrast to NK cells, MAIT cells do not display parallel increases in OxPhos (28). To link MAIT cell glycolytic metabolism to cytokine production, we used the glycolytic pathway inhibitor 2DG and show that at low doses it strongly inhibits MAIT cell production of IFN-γ. We also show that acute restriction of glucose during MAIT cell activation significantly impacted their activation, confirming their glycolytic requirements. Interestingly, 2DG treatment did not impact TNF-α production by MAIT cells, whereas treatment with oligomycin reduced TNF-α production but not IFN-γ. Previous studies in macrophages have described the differential regulation of TNF-α, showing that 2DG blocks IL-1β but not TNF-α. This suggests differential metabolic regulation of TNF-α production in MAIT cells.

We and others have previously reported that MAIT cells are defective in obese adults, although the mechanism(s) underpinning their dysregulation are unknown (8, 29). To elucidate the mechanisms driving the alterations in cytokine production, we performed RNA-Seq of MAIT cells isolated from lean and obese adults. We observed alterations in the expression over 400 genes between lean and obese donors, and principal component analysis

separated MAIT cells isolated from lean donors from MAIT cells isolated from obese donors. Pathway analysis revealed changes in several cellular metabolism pathways, including the downregulation of genes associated with glycolytic metabolism in MAIT cells isolated from obese individuals. To confirm our sequencing data, we performed a series of experiments, which show for the first time, to our knowledge, that glycolytic metabolism is defective in MAIT cells isolated from obese adults. This parallels our recent study showing defective glycolytic metabolism in NK cells from obese adults (30). In contrast to NK cells and the MAIT data from obese adults presented in this study, we previously reported that in a cohort of obese children there was altered NK cell metabolism with elevated rates of glycolysis, which may suggest that chronic activation of glycolytic machinery in childhood obesity results in a failure later in life (31).

The mTOR complex is highlighted both as an important signaling complex and regulator of metabolism in immune populations such as $CD8^+$ T cells and NK cells (20, 26, 32). Our data identify mTORC1 as a key regulator of MAIT cell metabolism. In this study, we show that MAIT cells increase mTORC1 activity upon activation, which is essential for the elevated levels of MAIT cell metabolism upon activation. Finally, our sequencing data highlighted reductions in mTORC1 signaling in MAIT cells isolated from obese individuals. We show diminished mTORC1 activation in MAIT cells but not classical $CD8^+$ T cells isolated from obese individuals as compared with lean controls. Previous studies show that inhibition of mTORC1 with rapamycin results in reduced effector immune cell functions (20, 33). Indeed, inhibition of mTORC1 activation with rapamycin inhibited IFN-γ production, which is in line with reduced rate of glycolysis observed in rapamycin-treated MAIT cells and the requirement of glycolysis for MAIT cell IFN-γ production. These findings contribute to our understanding as to why MAIT cell functional responses are impaired in obesity.

To investigate why mTOR activity is diminished in MAIT cells from obese donors, we investigated the upstream regulators of mTOR. Previous studies by our collaborators have extensively shown that AKT is dispensable for T cell metabolism and NK cell metabolism but highlight amino acid transport as an important regulator of mTORC1 activity (24, 26, 34, 35). To this end, we show that MAIT cell activation of mTORC1 and subsequent upregulation of glycolytic metabolism and IFN-γ production is dependent on amino acid transport into the cell. We also show that MAIT cells from obese donors have reduced expression of the system L amino acid transporter SLC7A5 and thus reduced amino acid uptake. Numerous studies have highlighted the dysregulation of obesity on amino acid metabolism, in particular branched chained amino acids such as leucine, which is the candidate amino acid implicated in the activation of mTOR in T cells (36–41).

Collectively, our data show that MAIT cells use glycolytic metabolism during their production of IFN-γ and that this process is regulated by mTORC1, which in turn is dependent on amino acid flux into the cell. In obesity, RNA-Seq of MAIT cells revealed downregulation of genes involved in both glycolytic metabolism and mTORC1 signaling. We confirmed these alterations using a series of in vitro experiments. These findings provide a mechanism for the reported alterations in MAIT cell IFN-γ production in obesity and may help us to understand the alterations of MAIT cells in other human diseases such as hepatitis C virus, malignant myeloma, and colorectal cancer (42–44). Finally, understanding the metabolic pathways controlling MAIT cell activation may highlight novel targets for controlling their functions in disease (45).

Disclosures

The authors have no financial conflicts of interest.

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