Received: 15 January 2025 | Accepted: 26 February 2025

DOI: 10.1097/HC9.0000000000000714

ORIGINAL ARTICLE

OPEN

Antioxidant interventions reduced cytokine-induced pyroptosis of peripheral MAIT cells in patients with HBV-related cirrhosis

Zheng Xu[1] | Zhe Xu[2] | Xing Fan[2] | Meng-Meng Qu[2] | Hongmin Wang[2] | Jiaying Li[2] | Lingyu Gao[3] | Yan-Mei Jiao[2] | Jijing Shi[3] | Fu-Sheng Wang[1,2]

[1]Department of Immunology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi, Xinjiang, China

[2]Senior Department of Infectious Diseases, The Fifth Medical Center of PLA General Hospital, National Clinical Research Center for Infectious Diseases, Beijing, China

[3]Key Medical Laboratory of Stem Cell Transformation and Application, The First People's Hospital of Zhengzhou, Zhengzhou, Henan, China

Correspondence
Yan-Mei Jiao, Senior Department of Infectious Diseases, The Fifth Medical Center of PLA General Hospital, Beijing 100039, China.
Email: jiaoyanmei@sina.com

Jijing Shi, Key Medical Laboratory of Stem Cell Transformation and Application, The First People's Hospital of Zhengzhou, Zhengzhou, Henan 450000, China.
Email: shijijing1983@126.com

Fu-Sheng Wang, Senior Department of Infectious Diseases, The Fifth Medical Center of PLA General Hospital, Beijing 100039, China.
Email: fswang302@163.com

Abstract

Background: Mucosal-associated invariant T (MAIT) cells are diminished in various liver diseases, but the underlying mechanism remains unclear. This study aimed to investigate the characteristics and underlying mechanisms of MAIT cell depletion in HBV-related cirrhosis.

Methods: Peripheral blood samples were collected from 20 healthy controls and 40 patients with HBV-related cirrhosis, divided into compensated (20) and decompensated (20) liver cirrhosis groups. Flow cytometry, single-cell RNA sequencing (scRNA-seq), multiplex immunofluorescence, and ELISA were used to assess MAIT cell characteristics.

Results: In patients with HBV-related cirrhosis, MAIT cells were significantly reduced and hyperactivated. The levels of pyroptosis and oxidative stress were elevated, particularly in those with decompensated liver cirrhosis (DLC). As disease severity increased, both pyroptosis and oxidative stress in MAIT cells rose, negatively correlating with MAIT cell frequency. Additionally, MAIT cells from patients with compensated liver cirrhosis (CLC) and DLC had lower levels of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), granzyme B (GZMB), and CD107a, but higher IL-17A levels. Blocking IL-12 and IL-18 pathways reduced MAIT cell activation and pyroptosis, while antioxidants effectively decreased pyroptosis in vitro.

Conclusions: Pyroptosis contributes to the decline of MAIT cells in HBV-related cirrhosis, while antioxidants can reduce this process.

Abbreviations: APC, activate antigen-presenting cell; CHB, chronic hepatitis B; CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis; FLICA, fluorochrome-labeled inhibitors of caspase; GO, Gene Ontology; GSDMD, gasdermin D; GZMB, granzyme B; HC, healthy control; HLA-DR, Human leukocyte antigen-DR; IFN-γ, Interferon-gamma; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC, liver cirrhosis; MAIT, mucosal-associated invariant T cell; MR1, major histocompatibility complex-I molecule; MTA, mitochondria-targeted antioxidants; PBMC, peripheral blood mononuclear cell; ROS, reactive oxygen species; scRNA-seq, single-cell RNA sequencing; TCR, T-cell receptor; TNF-α, tumor necrosis factor-alpha; UMAP, uniform manifold approximation and projection.

Keywords: antioxidant, cytokines pyroptosis, HBV-related cirrhosis, mucosal-associated invariant T cell

INTRODUCTION

HBV-related cirrhosis refers to the diffuse liver damage caused by the progression of chronic hepatitis B (CHB) infection into its advanced stages. The annual incidence of liver cirrhosis (LC) in CHB patients who have not received antiviral treatment ranges from 0.1% to 9.7% per 100 person-years.[1] When liver function continuously deteriorates, it can lead to serious complications, such as ascites and hepatic encephalopathy. These complications indicate that HBV-related cirrhosis has advanced from compensated liver cirrhosis (CLC) to decompensated liver cirrhosis (DLC), with an annual progression rate of about 15% over 5 years.[2–4] Once DLC is established, the 5-year survival rate plummets to a mere 14%.[5]

The liver serves as a vital immune organ within the human body, and there is a close link between liver diseases and the immune system. In HBV-related cirrhosis, many immune cells are activated, leading to significant changes in their quantity and functionality. These modifications can markedly influence the course of the disease.[6]

Mucosal-associated invariant T (MAIT) cells are a unique subset of innate-like T cells that were initially identified by Porcelli et al[7] in human intestinal mucosal lymphoid tissue. Subsequent studies revealed that MAIT cells constitute ~1%–10% of the total T cell population in peripheral blood, with their proportion of all T cells in the liver soaring up to 50%,[8] underscoring their critical role in liver immunity. MAIT cells possess semi-invariant αβ T-cell receptors (TCRs) that are capable of recognizing riboflavin (vitamin B2) metabolites derived from bacteria and presented by the major histocompatibility complex-I molecule (MR1).[9] The invariant nature of MR1 facilitates the swift activation of MAIT cells during the initial stages of the immune response.[10] In addition to MR1-dependent activation, MAIT cells can also be triggered by cytokines, such as IL-12 and IL-18, with other cytokines, including interferon-γ (IFN-γ), IL-7, and IL-15, further potentiating this activation.[11,12] Bacteria that cannot synthesize riboflavin can activate antigen-presenting cells (APCs) through toll-like receptor ligands (TLRLs), thereby providing an alternative activation pathway for MAIT cells. Upon activation, MAIT cells generate a range of effector molecules, including IFN-γ, tumor necrosis factor-α (TNF-α), IL-17A, granzyme B (GZMB), and CD107a, which play pivotal roles in immune responses and disease pathogenesis.[12,13]

Several studies have investigated the role of MAIT cells in liver disease. In alcoholic fatty liver disease, chronic and excessive alcohol intake increases intestinal permeability, impairing antibacterial defenses and leading to a reduction in MAIT numbers.[14] A similar decline in MAIT cells is observed in viral hepatitis. In patients with the HCV, MAIT cells in the liver are activated and subsequently depleted by the cytokine IL-18.[15] In the case of HBV, conjugated bilirubin can directly trigger MAIT cells' apoptosis and inhibit their proliferation upon activation by TCRs.[16] HCC is associated with a reduction in MAIT cells, which is attributed more to immune exhaustion than to apoptosis, with tumor cells expressing MR1 redirecting MAIT cells from an antitumor to a protumor stance.[17] Collectively, the diminished presence of MAIT cells is a recurring theme in chronic liver diseases, with their antibacterial capabilities and cytokine secretion compromised as the disease evolves. Gaining insights into the diverse mechanisms of MAIT cells' activation and demise across various diseases is essential for elucidating their involvement in liver disorders.

Pyroptosis is a form of programmed cell death characterized by the activation of caspase-1. This process cleaves gasdermin D (GSDMD), causing cell membrane rupture and the release of pro-inflammatory cytokines,[18] which play a crucial role in the innate immune response against pathogens.[19] Many innate-like T cells exhibit the characteristics of innate cells, such as natural killer T cells, which undergo pyroptosis during diseases.[20] MAIT, classified as innate-like T cells, also exhibit this characteristic. Research shows that in patients with HIV-1 infection and alcoholic liver disease, the markers of pyroptosis are significantly higher in MAIT cells.[21,22] However, it remains unclear whether MAIT cells undergo pyroptosis in HBV-related cirrhosis. Oxidative stress, caused by an imbalance of reactive oxygen species (ROS), can lead to cellular dysfunction or death and is considered a key factor in pyroptosis.[23] Overexpression of ROS may induce NLRP3 expression and impair lysosomal function, ultimately triggering cell pyroptosis.[24] We propose that MAIT cells undergo alterations in quantity and functionality and tend toward pyroptosis in HBV-related cirrhosis. Antioxidant interventions may partially alleviate MAIT pyroptosis, suggesting a potential therapeutic approach.

METHODS

Participants

A total of 60 participants were recruited from the Fifth Medical Center of the PLA General Hospital between March 2022 and February 2024. The participants

comprised 20 patients with CLC, 20 patients with DLC, and 20 healthy controls (HCs). The diagnosis of HBV-related cirrhosis was confirmed by establishing the presence of HBV infection along with cirrhosis, which was verified through either liver biopsy or clinical evidence. The clinical evidence included tests and examinations, such as clinical manifestations, laboratory tests, liver stiffness measurements, and imaging results. Decompensated cirrhosis was identified when cirrhosis was complicated by issues such as ascites, hemorrhage from gastroesophageal varices, sepsis, hepatic encephalopathy, or hepatorenal syndrome.[25] Exclusion criteria for the study were other types of hepatitis infections, liver cancer, metabolic liver diseases, rheumatic and connective tissue diseases, and steroid use. The patients' clinical characteristics are summarized in Table 1. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Fifth Medical Center of PLA General Hospital (Number: KY-2022-4-30-1). All participants provided informed consent before enrollment.

Antibodies and flow cytometric analysis

Fluorescence-conjugated reagents and antibodies were obtained from BioLegend, including anti-CD3-BV605 (OKT3), anti-TCRVα7.2-BV421 (3C10), anti-CD161-PE

TABLE 1 Participant characteristics

	CLCs (n = 20)	DLCs (n = 20)	*p*
Age (y)	61.15 (47, 77)	58.40 (46, 72)	0.327
Male/female	11/8	14/6	0.514
Regular blood test			
White blood cell ($\times10^9$/L)	4.22 (2.1, 7.74)	3.57 (1.52, 7.46)	0.183
Red blood cell ($\times10^9$/L)	4.34 (2.93, 6.01)	3.79 (1.92, 5.52)	0.108
Neutrophils ($\times10^9$/L)	2.26 (0.85, 4.19)	2.42 (0.72, 6.56)	0.779
Lymphocytes ($\times10^9$/L)	1.48 (0.60, 2.87)	1.04 (0.31, 2.34)	0.023
Monocytes ($\times10^9$/L)	0.28 (0.12, 0.43)	0.33 (0.09, 0.55)	0.265
Platelet ($\times10^9$/L)	129.00 (27, 215)	77.65 (23, 180)	0.005
Hemoglobin (g/L)	134.65 (84, 166)	110.50 (66, 161)	0.011
Coagulation indicators			
Prothrombin time (s)	12.29 (11.40, 14.40)	13.80 (11.10, 21.00)	0.006
International normalized ratio	1.11 (0.99, 1.68)	1.36 (0.96, 2.21)	0.002
Prothrombin time activity (%)	89.68 (40.40, 110.40)	69.93 (19.10, 117.70)	0.013
Liver function indicators			
Total bilirubin (μmol/L)	16.75 (8.20, 34.30)	40.36 (8.0, 165.60)	0.001
Direct bilirubin (μmol/L)	5.29 (2.50, 14.40)	18.88 (3.70, 98.70)	< 0.001
Indirect bilirubin (μmol/L)	11.46 (5.20, 19.90)	21.48 (4.30, 66.90)	0.060
Total bile acid (μmol/L)	12.50 (2.00, 47.00)	75.85 (2.00, 105.00)	< 0.001
ALT (U/L)	20.95 (9.00, 33.00)	41.40 (16.00, 129.00)	0.192
AST (U/L)	26.00 (18.00, 41.00)	27.90 (9.00, 66.00)	0.004
Albumin (g/L)	40.80 (29.00, 77.00)	32.15 (18.00, 42.00)	< 0.001
Blood ammonia (mmol/L)	35.87 (5.70, 90.00)	55.41 (17.40, 117.70)	0.018
Creatinine (μmol/L)	79.85 (56.00, 104.00)	77.80 (46.00, 172.00)	0.201
MELD score	7.60 (6.00, 9.00)	11.55 (6.00, 24.00)	< 0.001
Child–Pugh score	5.20 (5.00, 7.00)	6.95 (5.00, 11.00)	< 0.001
Virological indicators			
HBsAg (COI)	3215.20 (0.60, 7771.00)	2721.87 (0.51, 7269.00)	0.730
HBeAg (COI)	0.18 (0.07, 0.62)	29.69 (0.07, 489.60)	0.480
Inflammation indicators			
C-reactive protein (mg/dL)	1.68 (0.10, 6.94)	4.83 (0.10, 25.10)	0.201
Procalcitonin (ng/mL)	0.05 (0.03, 0.11)	0.16 (0.03, 0.46)	0.037
Interleukin-6 (pg/mL)	12.44 (1.40, 32.28)	19.42 (3.22, 95.51)	0.660

Note: Data are presented as median (IQR) or number (%).
Abbreviations: Child–Pugh score, Child–Pugh liver function score; CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis.

(W18070C), anti-CD38-BV605 (HIT2), anti-HLA-DR-Percp/Cyanine5.5 (L243), anti-GZMB-FITC (QA16A02), anti-CD107a-PE/Cyanine7 (1D4B), anti-TNF-α-BV510 (MAb11), anti-IL-17A-APC (BL168), and anti-IFN-γ-PE/Percp/Cyanine5.5 (4S.B3). The fluorochrome-labeled inhibitors of caspases (FLICA) caspase-1 reagent (Bio-Rad) was utilized to assess the pyroptosis of MAIT cells, and the ROS Assay Kit-Highly Sensitive DCFH-DA (Dojindo) was utilized to assess the levels of oxidative stress of MAIT cells. Fixable Viability Stain 700 (BD Biosciences) was used to distinguish viable from nonviable peripheral blood mononuclear cells (PBMCs).

The PBMCs were first stained using live/dead cell staining, followed by incubation with FLICA caspase-1 and DCFH-DA at 37 °C for 1 hour. Next, surface antibody staining was performed at 4 °C for 30 minutes. After washing the cells, they were permeabilized and fixed at 4 °C for 30 minutes. Finally, intracellular antibodies were added and incubated at 4 °C for 30 minutes. Data were analyzed using NovoExpress software (Acea Bioscience, Inc.) and FlowJo software (FlowJo).

Single-cell RNA sequencing (scRNA-seq) data processing, multiple dataset integration, and cell-type annotation

The raw FASTQ single-cell RNA sequencing (scRNA-seq) files from the liver tissues of 4 patients and 6 HCs were retrieved from the Genome Sequence Archive at the Beijing Institute of Genomics Data Center (http://bigd.big.ac.cn/gsa-human, accession HRA001730 and HRA000069) and from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov, accession RPRJNA833766). The downloaded reads were processed individually with the Cell Ranger count pipeline (v.4.0.0; 10x Genomics, https://www.10xgenomics.com), utilizing the GRCh38 human reference genome to generate gene expression matrices. The subsequent analyses were conducted using R scripts (v.4.0.2) with the Seurat package (v.3.2.2). Genes expressed in at least 0.1% of the cells were retained for each sample, while cells were filtered based on the following criteria: (1) a minimum of 500 genes detected; (2) a minimum of 800 unique molecular identifiers; and (3) mitochondrial gene expression not exceeding 10%. Cells failing to meet these criteria were excluded from further analysis. The datasets from different samples across four conditions were integrated into a unified dataset employing the standard workflow outlined at https://satijalab.org/seurat/v3.2/integration.html. The integrated dataset was scaled, followed by principal component analysis. The top 17 principal components were selected to construct a shared nearest neighbor network, and an unsupervised graph-based clustering approach utilizing the Louvain algorithm was applied, with a resolution parameter set to 0.5 for cell clustering purposes. The clusters were classified and annotated based on established marker genes. MAIT cells were identified using SLC4A10 as a marker encoding solute carrier family member 4, member 10, and extracted into another dataset for downstream analysis. Finally, uniform manifold approximation and projection (UMAP) was employed to visualize the clustering results in a 2-dimensional space.

Identification of differentially expressed genes and enrichment analysis of Gene Ontology and Kyoto Encyclopedia of Genes and Genomes

To identify differentially expressed genes (DEGs) across various clusters and conditions, the "Find Markers" function within the Seurat package was utilized with multiple threshold parameters, including an average log2 (fold change) ≥ 0.5, a Benjamini–Hochberg-corrected p value ≤ 0.01, and detection in at least 10% of cells in one condition. The identified DEGs were subsequently uploaded to the Metascape web tool (https://www.metascape.org), where gene sets derived from Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were selected to elucidate their functional profiles.

Multiplex immunofluorescence measurement

Control liver tissue samples ($n = 5$) were procured from deceased donors deemed suitable for liver transplantation, while liver tissues ($n = 5$) from patients with LC who underwent biopsies were obtained from the Fifth Medical Center of the PLA General Hospital and the First People's Hospital of Zhengzhou. The liver tissue sections (5 μm) were incubated with purified primary antibodies: anti-CD3 (EP41, 1:300; Zhongshan Golden Bridge Biotechnology), anti-MDR-1 (Ab170904, 1:280; Abcam), anti-human GSDMD (1:550; Abcam), and anti-NRF-2 (1:18,000; Proteintech) for 60 minutes at 37 °C before being washed with horseradish peroxidase-labeled secondary antibodies. After amplification using ×1 tyrosine signal amplification solution and DAPI staining, images were acquired using an Aperio VERSA scanning system (Leica).

Stimulation assay

The PBMCs were suspended in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and a 1% penicillin-streptomycin solution (100 IU/mL, 4A Biotech). Subsequently, the

cells were treated in U-bottomed 96-well plates at a density of 1×10^6 cells per well in a volume of 200 μL, under conditions of 37 °C and 5% CO_2. The treatments included IL-12 or IL-18 (PeproTech) added at a 200 ng/mL concentration. For cell stimulation and inhibition, the treatments comprised (1) medium only and (2) *Escherichia coli* DH5α fixed with 4% paraformaldehyde (PFA) at a ratio of 10 bacteria per cell, either in the absence or presence of an MR1-blocking antibody (clone 26.5), IL-12p40/70-blocking antibody (5 μg/mL, eBioscience), or IL-18-blocking antibody (20 μg/mL, R&D Systems). For the intervention with antioxidants, the treatments included the following: (1) medium only, (2) the addition of IL-12 or IL-18 at a concentration of 200 ng/mL, and (3) the same combination supplemented separately with polyphenols (ie, resveratrol [5 μM, APExBIO] and oleuropein [0.25 μM, APExBIO]); iACAT (ie, avasimibe [0.25 μM, Selleck], and K-604 [0.05 μM, MCE]; and mitochondria-targeted antioxidants (MTA) (ie, Mito-TEMPO [5 μM, Merk] and MitoQ [0.05 μM, MCE]). All cells were cultivated for 24 hours.

ELISA

The soluble IL-12p70 (R&D Systems) and IL-18 kits (R&D Systems) were utilized to quantify the plasma concentrations of IL-12p70 and IL-18, strictly adhering to the manufacturer's protocols. Each sample was analyzed in duplicate to ensure accuracy and reproducibility.

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics version 21. The distribution characteristics of the data were assessed using the Kolmogorov–Smirnov normality test. For 2 groups of non-normally distributed variables, nonparametric Mann–Whitney *U* tests were employed, while the Wilcoxon signed-rank test was utilized for matched pairs. Correlations between variables were evaluated using the Spearman rank correlation coefficient. Statistical significance was defined as $p<0.05$, with $^*p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ indicating increasing significance levels.

RESULTS

The frequencies of MAIT cells decrease with the severity of HBV-related cirrhosis

We analyzed the frequency of peripheral MAIT cells in 20 patients with CLC, 20 patients with DLC, and 20 HCs. The MAIT cells in the PBMCs were identified as $CD3^+V\alpha7.2^+CD161^{high}$ cells. The frequency of MAIT cells in patients with HBV-related cirrhosis was significantly lower than that observed in the HCs (Figures 1A, B). Additionally, when comparing the patients with CLC to those with DLC, a marked reduction in MAIT numbers was noted among the latter.

Immunofluorescence imaging revealed a decreased density of MAIT cells (marked by $CD3^+MDR\text{-}1^+$)[21] in the liver tissues of the patients with CLC and DLC than in the HCs (Figure 1C). The correlation analysis results showed that the frequency of MAIT cells was negatively correlated with the clinical indicators that represent disease severity (ie, the Child–Pugh liver function score, the MELD score, and total bile acid levels) (Figure 1D).

MAIT cells are activated, undergo pyroptosis, and have their functionality impaired in HBV-related cirrhosis

We evaluated MAIT cells' activation and pyroptosis along with the expression levels of related functional factors during HBV-related cirrhosis. We found that the peripheral MAIT cells in patients with HBV-related cirrhosis exhibited elevated levels of HLA-DR and CD38 compared to the HCs. Additionally, the expression of HLA-DR and CD38 was significantly higher in the patients with DLC than in those with CLC (Figures 2A, B). FLICA caspase-1 and intracellular staining with antibodies were used to specifically recognize the active forms of caspase-1 and caspase-3.[26,27] The MAIT cells in the patients with HBV-related cirrhosis displayed increased levels of FLICA caspase-1 and were particularly pronounced in the patients with DLC (Figure 2C). Further analysis revealed that the expression levels of FLICA caspase-1 exhibited a positive correlation with the severity of liver disease, as indicated by the Child–Pugh score, MELD score, and total bile acid concentrations (Figure 2I).

Regarding the functional changes of MAIT cells in HBV-related cirrhosis, we detected representative key cytokines in the PBMCs, including pro-inflammatory cytokines, such as IFN-γ and TNF-α, cytotoxic and antibacterial molecules, such as CD107a and GZMB, and the tissue repair cytokine IL-17A. The expression of IFN-γ, TNF-α, CD107a, and GZMB was diminished in the disease groups compared to the HCs, and particularly pronounced in the patients with DLC, where these intracellular proteins exhibited notable downregulation (Figures 2D–G). Interestingly, there was an opposing trend in the expression of IL-17A compared to other intracellular factors, showing an increase in the disease group, especially in the patients with DLC (Figure 2H). These data suggest that the ability of MAIT cells to secrete intracellular factors generally declines during the progression of HBV-related cirrhosis.

scRNA-seq analyses reveal the transcriptional characterization of MAIT cells in patients with HBV-related cirrhosis

Single-cell transcriptomic data from 3 previous studies were integrated to characterize the phenotypic and molecular features of MAIT cells during HBV-related cirrhosis.[28–30] We obtained 65487 immune cells derived from healthy livers (n = 6) and livers with HBV-related cirrhosis (n = 4). UMAP and unsupervised graph-based clustering partitioned the cells into 7 clusters based on the most significant marker genes (Figures 3A, C). Based on their transcriptional programs and surface protein expression profiles, 5 $CD8^+$ (C1, C2, C7, C10, and C12) and 3 $CD4^+$ (C3, C6, and C8) T cell subpopulations were identified along with natural killer cells (C0), γδT cells (C4), B cells (C9), myeloid cells (C11), plasma cells (C13, C14), and MAIT cells (C5). Specifically, 6698 MAIT cells were identified from the HCs and the patients with LC based on the expression levels of the canonical marker SLC4A10 (Figure 3B).

To comprehensively investigate the transcriptome characteristics of MAIT cells, we initially compared the gene expression profiles of MAIT cells with those of other immune cells and identified 1924 DEGs. Upon comparison of these DEGs, we found that 72 molecules were significantly upregulated, while 20 were notably downregulated. The upregulated proteins and genes included specific MAIT markers such as SLC4A10 and IL18RAP; T cell activation markers including THEMIS, NRIP1, and DPP4; inflammation-related markers such as IL18R1, IL18RAP, TNF, and LTB; cell death indicators such as TMEM117; and the endoplasmic reticulum stress marker ERN1 (Figure 3D). GO and KEGG enrichment analyses further revealed that the genes within MAIT cells were significantly enriched in pathways related to cell activation (Regulation of T cell activation, Immune response-activating signaling pathway, T cell receptor signaling pathway), ROS regulation pathway (Response to oxidative stress, Cellular response to oxidative stress, Chemical carcinogenesis reactive oxygen species), and cell death (Intrinsic apoptotic signaling pathway, Regulation of apoptotic signaling pathway, Extrinsic apoptotic signaling pathway, Apoptosis) (Figure 3E).

Next, we compared MAIT cells between the HC and LC groups, identifying 23 upregulated and 75 downregulated genes (Figure 3F). The upregulated proteins and genes of MAIT cells in HBV-related cirrhosis included cell death markers such as IGFBP3 and PTPN13; ROS regulation markers such as SDR42E2; mediators of adaptive immunity such as CTLA4; mediators of IL-17 secretion such as LEF1; and facilitators of

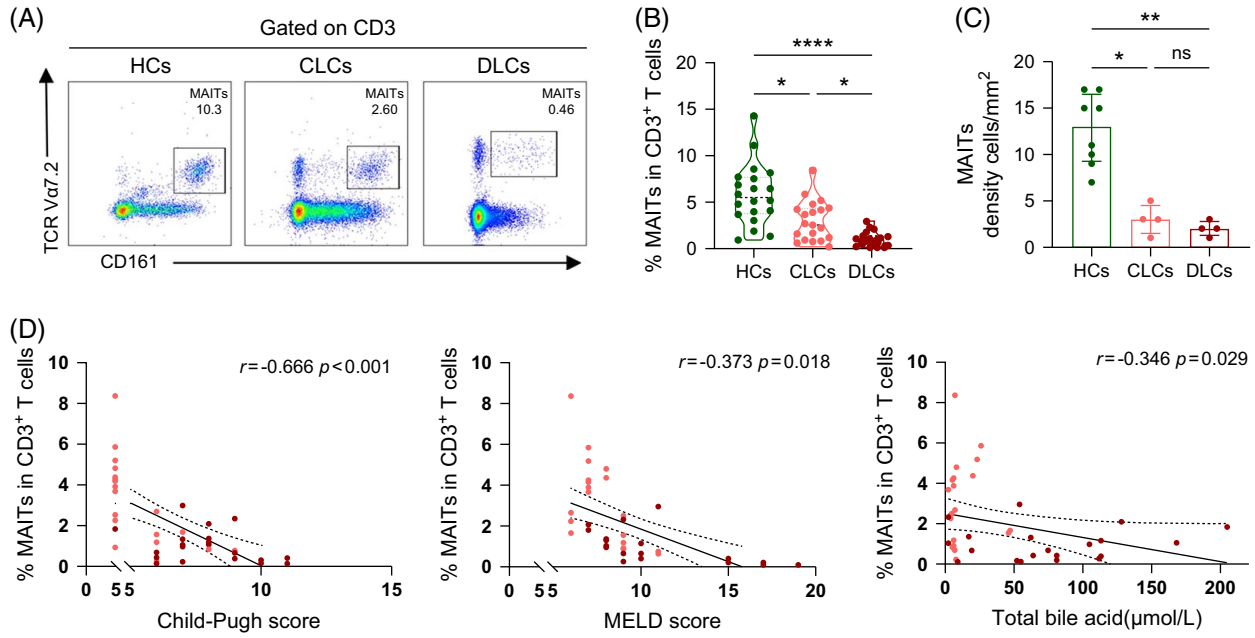

FIGURE 1 The frequency of mucosal-associated invariant T (MAIT) cells decreased with the severity of HBV-related cirrhosis. (A) Representative fluorescence-activated cell sorting (FACS) plots of circulating MAIT cells' frequencies ($TCRVa7.2^+$ $CD161^{high}$) gated on $CD3^+$ T cells from one healthy control (HC), one patient with compensated liver cirrhosis (CLC), and one patient with decompensated liver cirrhosis (DLC). (B) Violin plots showing MAIT cell frequencies in peripheral blood from HCs, patients with CLC, and patients with DLC detected by flow cytometry. The frequencies of MAIT cells in patients with DLC were significantly lower than in patients with CLC. (C) Summary of the density information of $CD3^+MDR\text{-}1^+$ in the liver tissues of HCs and patients with LC. (D) Correlation analysis of MAIT cell frequencies and Child–Pugh scores, MELD scores, and level of total bile acid was calculated in patients with CLC and DLC. (B, C) Nonparametric Mann–Whitney *U* tests. (D) Spearman rank correlation test. $^*p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$; ns, non-significant. Abbreviations: LC, liver cirrhosis; CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis; HC, healthy control; MAIT, mucosal-associated invariant T cell; Child–Pugh scores, Child–Pugh liver function score.

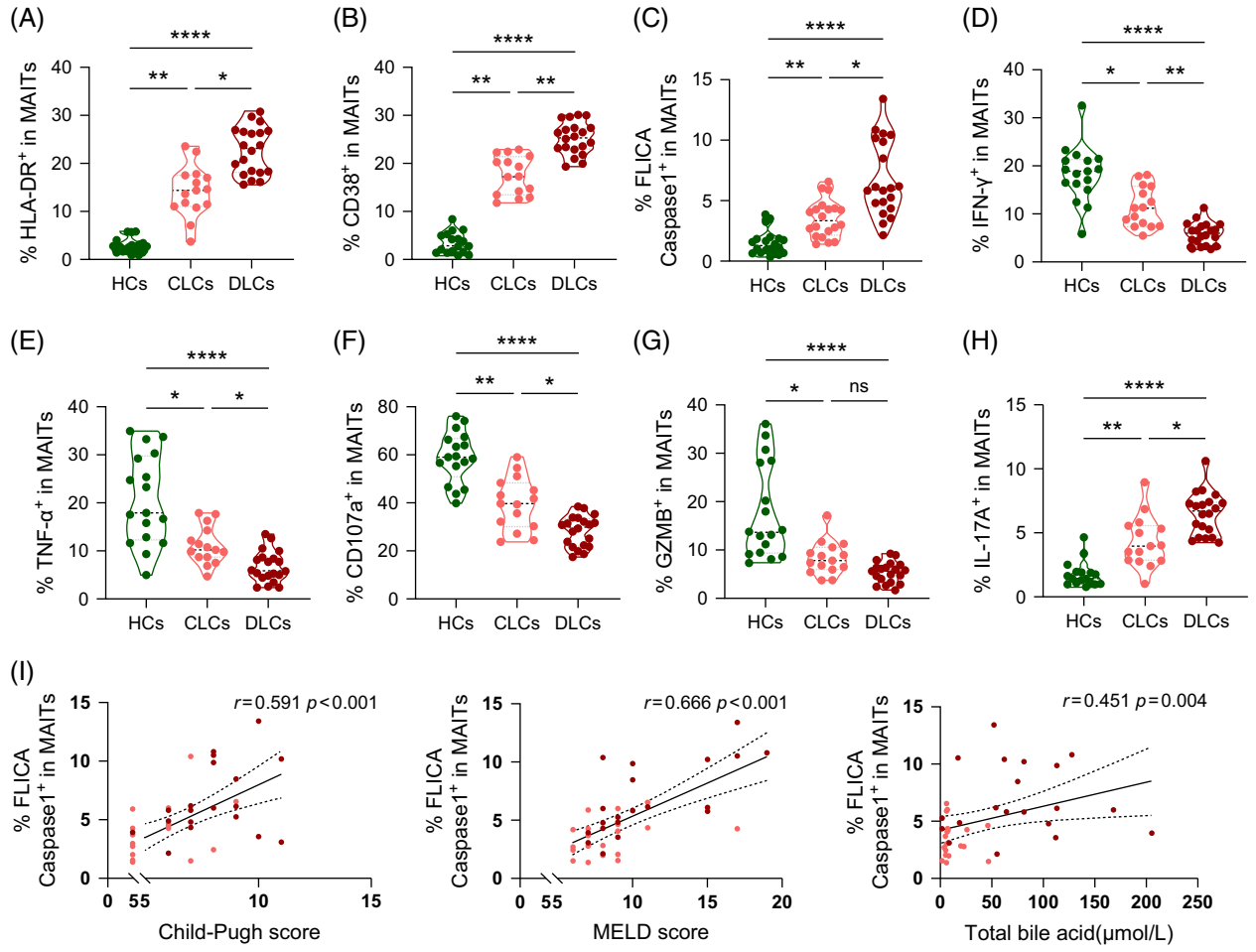

FIGURE 2 Mucosal-associated invariant T (MAIT) cells are activated, undergo pyroptosis, and have their functionality impaired in HBV-related cirrhosis. (A–H) Violin plots showing the frequencies of HLA-DR, CD38, and FLICA caspase-1 in the MAIT cells from the healthy controls (HCs) and patients with compensated liver cirrhosis (CLC) and decompensated liver cirrhosis (DLC) detected by flow cytometry. (D–H) Violin plots showing peripheral blood mononuclear cells freshly isolated from HCs and patients with CLC and DLC were stimulated with IL-12 and IL-18 (200 ng/mL for each) for 24 hours and then assessed for the expression level of interferon-γ, tumor necrosis factor-α, CD107a, granzyme B, and IL-17A in the MAIT cells. (I) Correlation analysis of pyroptotic MAIT cell frequencies and Child–Pugh scores, MELD scores, and level of total bile acid was calculated in patients with CLC and DLC. (A–H) Nonparametric Mann–Whitney *U* tests. (I) Spearman rank correlation test. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-signifcant. Abbreviations: CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis; HC, healthy control; MAIT, mucosal-associated invariant T cell; Child–Pugh scores, Child–Pugh liver function score.

cell adhesion such as THBS4-AS1, ADAM23, and PDZD2. The downregulated proteins and genes of MAIT cells in HBV-related cirrhosis included cytokine secretion markers such as IFNG, CSF1, and CD70; cell chemotaxis and adhesion markers such as CCL3 and PECAM1; and lipid metabolism markers such as LIPC, OSBPL5, and SCD. GO and KEGG enrichment analyses indicated that MAIT cells from the LC group were associated with IL-17A production (Th17 cell differentiation), ROS regulation pathway (Regulation of reactive oxygen species metabolic process, Reactive oxygen species metabolic process, Cellular response to oxidative stress, Chemical carcinogenesis reactive oxygen species, Response to mitochondrial depolarization), and cell death pathways (Intrinsic apoptotic signaling pathway, Extrinsic apoptotic signaling pathway, Apoptosis, Necroptosis) (Figure 3G). These data illustrate the genetic characteristics of MAIT cells in HBV-related cirrhosis.

The proportion of MAIT cells experiencing oxidative stress and their expression is associated with pyroptosis

To further validate the findings from the scRNA-seq analysis, we evaluated oxidative stress in MAIT cells across different cohorts and employed the mean fluorescence intensity of DCFH-DA Mean Fluorescence Intensity (MFI) as a surrogate marker for ROS expression in MAIT cells. We found elevated DCFH-DA MFI levels in the MAIT cells of the patients with

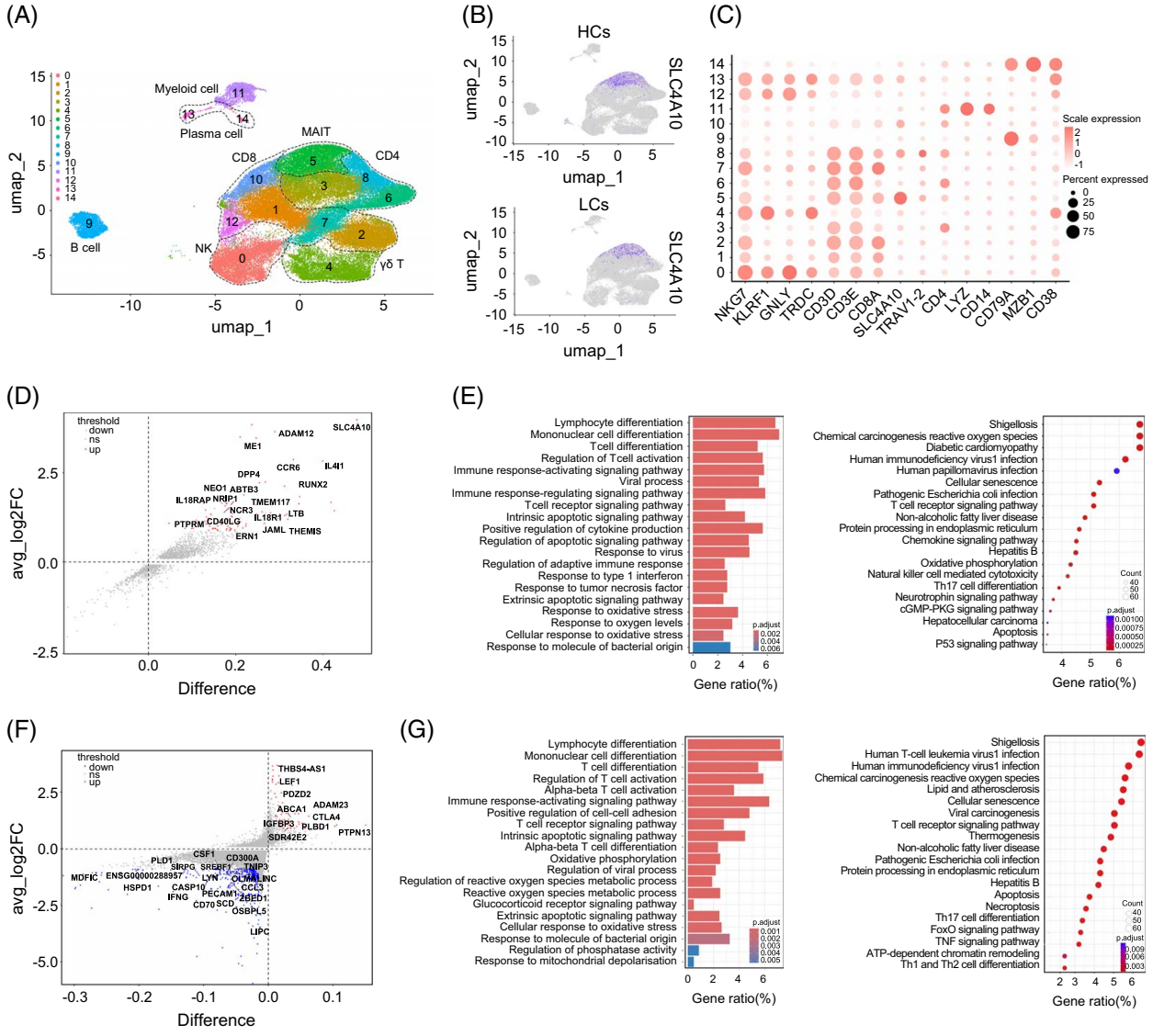

FIGURE 3 The single-cell RNA sequencing analyses revealed the transcriptional characterization of mucosal-associated invariant T (MAIT) cells in patients with HBV-related cirrhosis. (A) Uniform manifold approximation and projection plot of 65487 T cells from 6 healthy controls (HCs) and 4 patients with liver cirrhosis (LC) determined by Seurat v. 3. A total of 15 clusters (clusters 0–14) were identified and color-coded. (B) Dot plot illustrating the expression of canonical markers of MAIT cells from the HCs and the patients with LC. (C) Dot plots showing the expression of marker genes in each cluster. (D) Volcano plot showing the differentially expressed genes and surface proteins between the MAIT cells and the other immune cells. (E) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for genes comparing the MAIT cells to the other immune cells. (F) Volcano plot showing the differentially expressed genes and surface proteins between MAIT cells from the HCs and the patients with LC. (G) GO and KEGG enrichment analyses for genes in the MAIT cells comparing the HCs to the patients with LC. Abbreviations: GO, Gene Ontology; HC, healthy control; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC, liver cirrhosis; UMAP, uniform manifold approximation and projection.

HBV-related cirrhosis compared to the HCs, with significantly higher levels observed in the patients with DLC than those with CLC (Figures 4A, B). The correlation analysis indicated that the expression of DCFH-DA MFI was positively correlated with the expression levels of FLICA caspase-1 in the MAIT cells (Figure 4C). Subsequently, multicolor immunofluorescence staining of the liver tissue from the LC patients revealed that the MAIT cells ($CD3^+MDR\text{-}1^+$) exhibited markers of oxidative stress ($NRF\text{-}2^+$)[31] and pyroptosis ($GSDMD^+$) (Figure 4D). These results confirm that MAIT cells undergo oxidative stress and pyroptosis in HBV-related cirrhosis.

Exposure to *E. coli* triggers the activation and pyroptosis of MAIT cells, with this stimulation mediated by cytokines

To clarify the key factors that induce oxidative stress and pyroptosis in MAIT cells, we stimulated PBMCs from patients with HBV-related cirrhosis and HCs

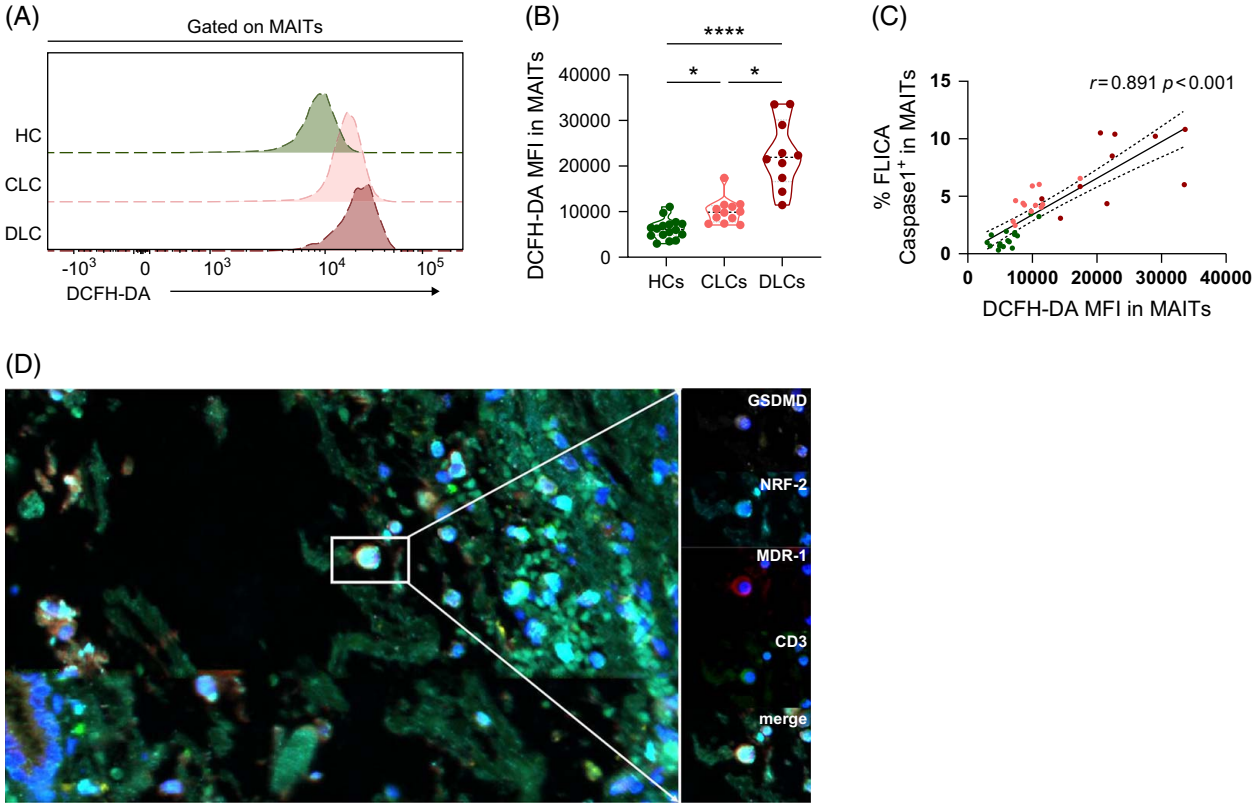

FIGURE 4 The proportion of mucosal-associated invariant T (MAIT) cells under oxidative stress increased with the severity of the patients. (A) Representative offset plots of DCFH-DA on the MAIT cells in the healthy controls (HCs), the patients with CLC, and the patients with DLC. (B) The MFI of DCFH-DA from the HCs, the patients with CLC, and the patients with DLC was detected by flow cytometry. (C) Correlation analysis of pyroptotic MAIT cells' frequencies and the level of DCFH-DA MFI in the MAIT cells. (D) MAIT cells ($CD3^+MDR\text{-}1^+$) in the liver tissue of the patients with HBV-related cirrhosis were co-stained with GSDMD and NRF-2. (B) Nonparametric Mann–Whitney *U* tests. (C) Spearman rank correlation test. $^*p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Abbreviations: CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis; GSDMD, gasdermin D; HC, healthy control; MAIT, mucosal-associated invariant T cell; MDR-1, Multidrug resistance gene-1; MFI, mean fluorescence intensity; NRF-2, nuclear factor erythroid 2-related factor 2.

in vitro using PFA-fixed *E. coli* DH5α. We then separately blocked IL-12/IL-18 and MR1 to elucidate the primary activation pathways. The results demonstrated significant upregulation of CD38 and HLA-DR in the MAIT cells (Figures 5A, B). In addition, compared to the control medium, there was a marked increase in DCFH-DA MFI and FLICA caspase-1 expression in the MAIT cells stimulated by PFA-fixed *E. coli* DH5α (Figures 5C, D). Blocking IL-12/IL-18 and MR1 allowed us to determine which pathway played a decisive role in MAIT activation under experimental conditions. Blocking MR1 did not alter the expression levels of CD38, HLA-DR, DCFH-DA MFI, or FLICA caspase-1 (Figures 5E–H). However, when IL-12/IL-18 was inhibited, the expressions of CD38, HLA-DR, DCFH-DA MFI, and FLICA caspase-1 were significantly downregulated (Figures 5A–D). The plasma levels of IL-12 and IL-18 were measured in the subjects. The expression of IL-12 was higher in patients with DLC compared to HCs. Furthermore, IL-18 levels were notably elevated in patients with HBV-related cirrhosis compared to HCs, and patients with DLC exhibited higher IL-18 expression than those with CLC (Figure 5I), suggesting that IL-12/18 constitutes the crucial factors mediating the activation and depletion of MAIT cells in HBV-related cirrhosis.

Antioxidants reduce cytokine-induced pyroptosis of MAIT cells

Given that cellular oxidative stress may be an upstream event in the pyroptosis process,[24,32] we aimed to regulate pyroptosis by applying antioxidants and assessing whether reducing oxidative stress levels could facilitate MAIT cells' recovery. Three types of antioxidants and related inhibitors were used as interventions: polyphenols from plants known for their extensive antioxidant properties, MTA, and the inhibitor of acetyl-CoA carboxylase transferase (iACAT) to modulate the MAIT cells activated by IL-12/18. Following the interventions, the DCFH-DA and FLICA caspase-1 levels in the MAIT cells were significantly decreased compared with those in the IL-12/18-stimulated group (Figures 6A, B). Among the comparisons between the 3 different antioxidants, the expression of

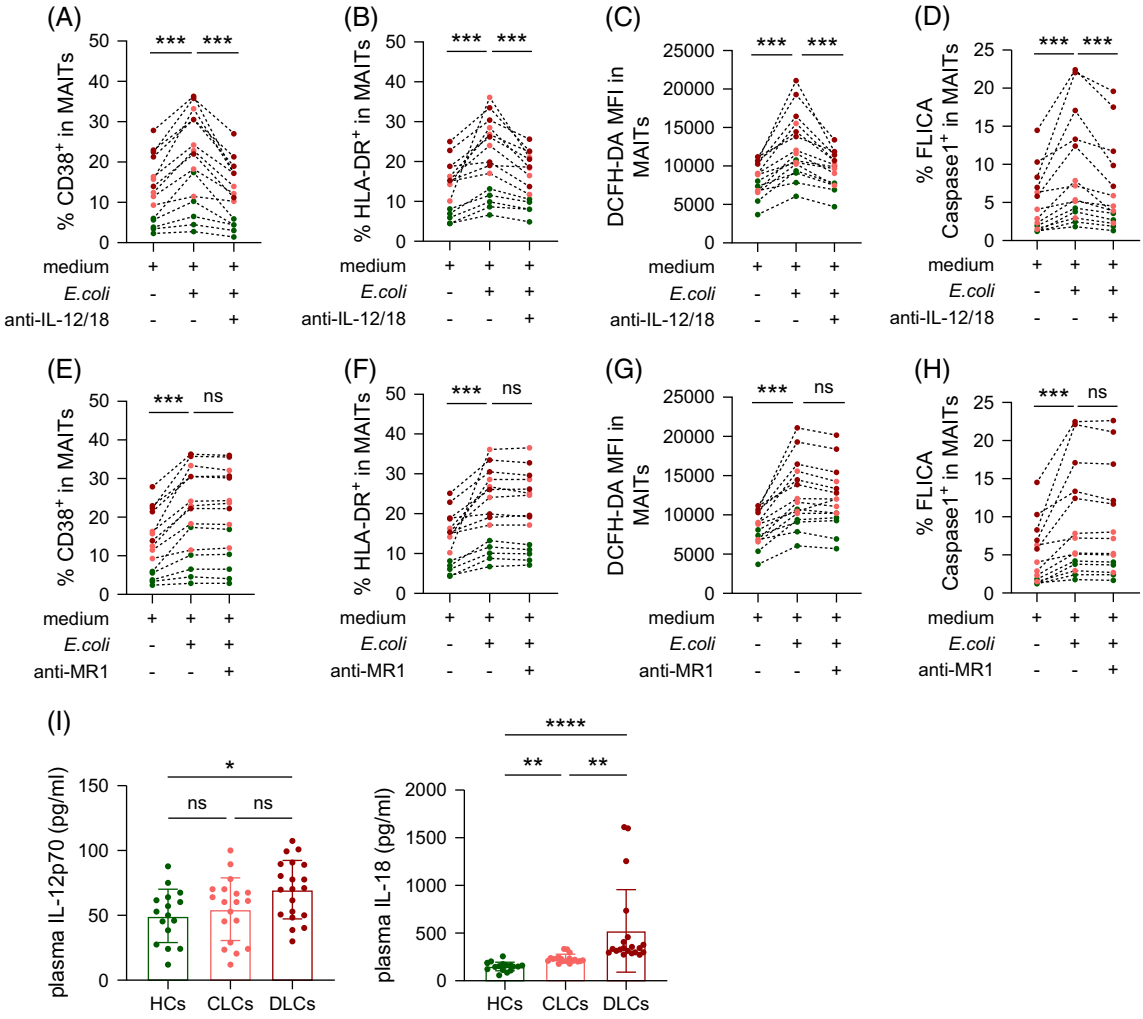

FIGURE 5 Exposure to *Escherichia coli* induced mucosal-associated invariant T (MAIT) cells' activation and pyroptosis, and this stimulation is achieved by cytokines. (A–D) Peripheral blood mononuclear cells (PBMCs) from healthy controls (HCs), patients with compensated liver cirrhosis (CLC), and patients with decompensated liver cirrhosis (DLC) were treated with medium only, *E. coli* and *E. coli* combined with anti-IL-18 or anti-IL-12 antibodies for 24 hours, respectively. The levels of CD38, HLA-DR, DCFH-DA MFI, and FLICA caspase-1 expressed in the MAIT cells were determined by flow cytometry. (E–H) PBMCs from HCs, patients with CLC, and patients with DLC were treated with medium only, *E. coli*, and *E. coli* combined with anti-MR1 antibody for 24 hours, respectively. The levels of CD38, HLA-DR, DCFH-DA, and FLICA caspase-1 expressed on the MAIT cells were determined by flow cytometry. (I) The level of IL-12p70 and IL-18 in the plasma was detected by ELISA. (A–I) Wilcoxon signed-rank test. $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$; ns, non-signifcant. Abbreviations: CLC, compensated liver cirrhosis; DLC, decompensated liver cirrhosis; HC, healthy control; MAIT, mucosal-associated invariant T cell; MFI, mean fluorescence intensity.

FLICA caspase-1 was higher in the MTA-treated group than in the polyphenol-treated group and the iACAT-treated group. Considering the potential impact of these antioxidants on functionality, we further evaluated cytokine and protein secretion by the MAIT cells. While the secretion levels of IFN-γ, TNF-α, CD107a, GZMB, and IL-17A in the MAIT cells were diminished following the antioxidant interventions compared to the IL-12/18-stimulated group, these levels remained significantly elevated when compared with those in the medium-only group (Figures 6C–G). Notably, among the tested antioxidants, the MTA-treated MAIT cells exhibited higher levels of IFN-γ, TNF-α, CD107a, GZMB, and IL-17A than those treated with polyphenol or iACAT. These results suggest that MTA can reduce oxidative stress and pyroptosis while maintaining the proper functioning of MAIT cells.

DISCUSSION

MAIT cells represent a distinctive subset of T lymphocytes that exhibit characteristics of both innate and adaptive immunity.[33] Their presence in the liver highlights their potential involvement in liver disease. Studies have shown that MAIT cells are significantly reduced in chronic inflammatory diseases, with their decline becoming more pronounced as the disease progresses.[34] In this study, we found that MAIT cells are highly activated and undergo pyroptosis in HBV-

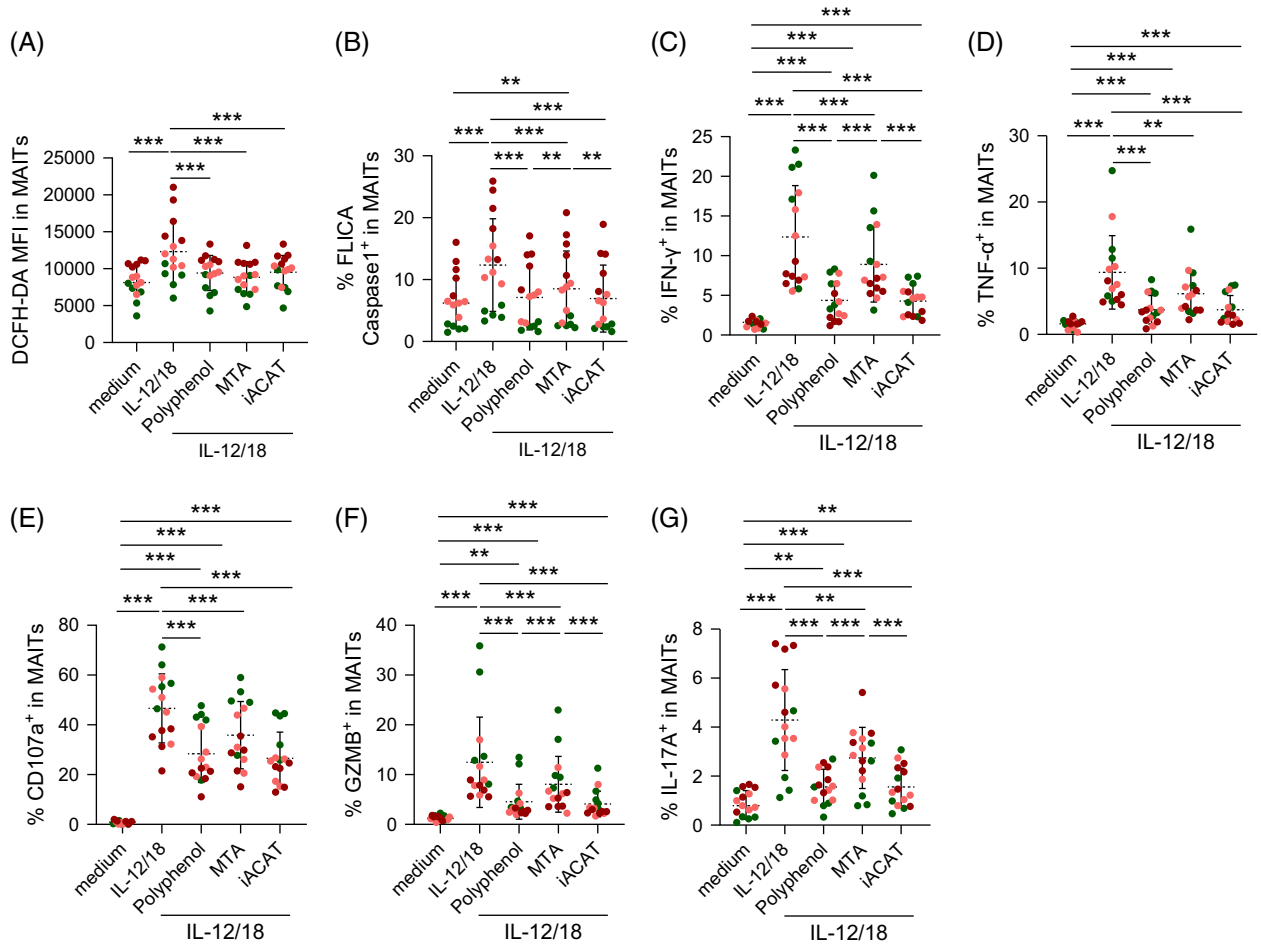

FIGURE 6 Antioxidants reduced the cytokine-induced pyroptosis of mucosal-associated invariant T (MAIT) cells. (A–G) Peripheral blood mononuclear cells from healthy controls, patients with compensated liver cirrhosis, and patients with decompensated liver cirrhosis were treated with IL-18 or IL-12 stimulants with polyphenols, MTA, or iACAT for 24 hours, respectively. The levels of DCFH-DA MFI, FLICA caspase-1, IFN-γ, TNF-a, GZMB, CD107a, and IL-17A expressed in the MAIT cells were determined by flow cytometry. (A–G) Wilcoxon signed-rank test. *$p < 0.05$, ***$p < 0.001$, and ****$p < 0.0001$, ns, non-signifcant. Abbreviations: MFI, mean fluorescence intensity; polyphenols, resveratrol and oleuropein; MTA, Mito-TEMPO and MitoQ; iACAT, Avasimibe and K-604.

related cirrhosis, particularly in DLC. This pyroptosis correlates with the disease severity, and similar findings have been observed in HIV-1 infection and alcoholic cirrhosis,[21,22] suggesting that pyroptosis may contribute to MAIT loss. Our scRNA-seq analysis indicated that MAIT cells were more activated and proliferative than other immune cells. In patients with HBV-related cirrhosis, MAIT cells showed higher oxidative stress and increased markers of cell death compared to the HCs, suggesting a swift response to infection and a tendency for rapid programmed cell death. Why do MAIT cells prefer pyroptosis over apoptosis? This may be characteristic of immune-like T cells. During pathogen invasion, MAIT pyroptosis helps eliminate bacteria and promote inflammation, which can activate other T cells.[35] In HBV-related cirrhosis, chronic inflammation leads to cytokine release, mitochondrial dysfunction, and ROS. This accumulation of ROS activates the NLRP3 inflammasome, triggering pyroptosis.[24] We hypothesized that high ROS levels in MAIT cells during HBV-related cirrhosis are closely linked to their pyroptotic response.

Under healthy conditions, ROS are vital for cellular signaling, immune defense, and metabolic balance. However, an excess of ROS can cause oxidative stress, which is associated with the onset and progression of diseases such as cancer, neurodegenerative disorders, cardiovascular diseases, and diabetes.[36] Our research shows that MAIT cells undergo oxidative stress and pyroptosis in HBV-related cirrhosis, with a significant correlation between the two. Consequently, we anticipate that the pyroptosis of MAIT cells could be modulated by employing antioxidant strategies.

Excessive oxidative stress in cells can be managed through strategies such as using antioxidants, activating endogenous antioxidant enzymes, and inhibiting ROS-generating enzymes.[37] Among these approaches, antioxidants provide a convenient and rapid intervention. To explore the effects of oxidative stress on pyroptosis in

MAIT cells, 3 antioxidants were used: polyphenols and MTA, which directly target mitochondrial function,[38] and iACAT, which indirectly affects mitochondrial activity by regulating fatty acid oxidation. Our results showed that antioxidants can reduce pyroptosis in MAIT cells, possibly compensating for their loss in HBV-related cirrhosis contexts. Recent studies have emphasized that oxidative stress is a key driver of apoptosis, pyroptosis, and ferroptosis.[23,24] Notably, its impact on ferroptosis and pyroptosis is particularly pronounced.[39,40] Certain stimuli primarily promote pyroptosis by activating the NLRP3 inflammasome,[24] while antioxidants such as polyphenols effectively inhibit this process,[41] which is consistent with the inhibitory effects we have observed. Furthermore, our results also indicate that in addition to polyphenols, the antioxidants MTA and iACAT can both effectively reduce pyroptosis in MAIT cells. Some findings suggest that MTA can alleviate oxidative stress by improving mitochondrial function,[38] while iACAT mainly indirectly affects oxidative stress by regulating cholesterol metabolism.[42] However, their specific mechanisms of action within cells still require further exploration. Although some studies have suggested that antioxidants might suppress cytokine secretion during immune regulation,[43,44] we found that MTA significantly alleviates oxidative stress and pyroptosis in MAIT cells while preserving their functional capabilities. It is noteworthy that MTA has already been used in clinical trials, with ample documentation on its safety and efficacy.[38] In particular, compared to non-targeted cell antioxidants, MTA has been shown to exhibit a greater protective effect. MitoQ is currently the only commercially available MTA (provided as a dietary supplement).[38] Whether supplementation with MitoQ can improve pyroptosis in MAIT cells and subsequently increase their numbers in patients with HBV-related cirrhosis will be the focus of our next research direction.

To study the activation of MAIT cells and pyroptosis, *E. coli* was used to stimulate MAIT cells in vitro,[45] with the hypothesis that MAIT cells would be activated under disease conditions, leading to oxidative stress and pyroptosis. The cytokines IL-12 and IL-18 play an important role in activating MAIT cells,[46] which are involved in this process. As supported by previous studies, the activation and demise of MAIT cells in CHB and alcoholic liver disease were predominantly mediated by cytokines,[47] suggesting a distinct role for cytokines in MAIT regulation compared to that of TCRs. In stark contrast, there was a marked upregulation of the pro-inflammatory cytokine IL-17A. IL-17A has been implicated in facilitating the transition from liver fibrosis to cirrhosis by stimulating the activation of HSCs, thereby exacerbating the severity and prognosis of liver pathologies.[48] This may be due to the immune environment influencing the functional phenotype shift of MAIT cells in HBV-related cirrhosis, or MAIT cells expressing IL-17A may be resistant to pyroptosis. This aligns with the characteristics of MAIT cells seen in other chronic diseases. In adult patients with type 1 diabetes, MAIT cells show reduced production of IL-2, IFN-γ, and TNF-α, while IL-17 and IL-4 levels are increased.[49] Additionally, research conducted by Hegde et al[50] indicated that MAIT cells from patients with cirrhosis are inclined to secrete higher amounts of IL-17, potentially exacerbating fibrosis. These functional modifications in MAIT cells suggest that their roles in disease pathogenesis may be more complex and variable than previously understood.

In HBV-related cirrhosis, we observed a substantial reduction in MAIT cells, accompanied by functional impairment and pyroptosis, which is strongly correlated with disease severity. Cytokines play a pivotal role in the activation and pyroptotic response of MAIT cells. There is a close association between MAIT cells' oxidative stress and pyroptosis, and we found that the use of antioxidants can significantly mitigate pyroptosis in these cells. However, given that our investigation was conducted in vitro, it remains uncertain whether antioxidants would exert a similar effect on MAIT cells' pyroptosis in vivo in patients suffering from HBV-related cirrhosis. This uncertainty will guide our forthcoming research endeavors.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

Zheng Xu: wrote the original draft, conducted experimental operations, and performed data analysis and organization. Meng-Meng Qu, Jiaying Li, Hongmin Wang, and Lingyu Gao: conducted experimental operations. Jijing Shi and Zhe Xu: collected and provided samples. Xing Fan, Yan-Mei Jiao, Jijing Shi, and Fu-Sheng Wang: review and editing, funding acquisition, and conceptualization.

ACKNOWLEDGMENTS

We thank Yiyun Li for his assistance with scRNA-seq data analysis, and we also appreciate Jinhong Yuan and Chunbao Zhou for their expertise in flow cytometry techniques. Special thanks are extended to Jiyuan Zhang for his contribution to this research.

FUNDING INFORMATION

This work was supported by grants from the National Key R&D Program of China (2023YFC2306800), the Young and Middle-aged Health Science and Technology Innovation Talent Project of Henan Province (YXKC2020060), the Science and Technology Development Plan Project of Henan Province (232102310021), and the National Key Research and Development Program (2022YFA1303603).

CONFLICTS OF INTEREST

The authors have no conflicts to report.

ORCID

Zheng Xu https://orcid.org/0009–0000–3064–0285
Zhe Xu https://orcid.org/0000–0002–3338–4229
Meng-Meng Qu https://orcid.org/0000–0001–6158–9443
Yan-Mei Jiao https://orcid.org/0000–0002–2556–326X
Fu-Sheng Wang https://orcid.org/0000–0002–8043–6685

REFERENCES

1. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: Special emphasis on disease progression and prognostic factors. J Hepatol. 2008;48:335–52.
2. Xu B, Hu DC, Rosenberg DM, JIANG QW, LIN XM, LU JL, et al. Chronic hepatitis B: A long-term retrospective cohort study of disease progression in Shanghai, China. J Gastroenterol Hepatol. 2003;18:1345–52.
3. Fattovich G, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: A cohort study of 297 patients. Am J Gastroenterol. 2002;97:2886–95.
4. Liaw YF, Sung JJY, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351:1521–31.
5. Guan R, Lui HF. Treatment of hepatitis B in decompensated liver cirrhosis. Int J Hepatol. 2011;2011:918017.
6. You H, Wang F-S, Li T, Xu X, Sun Y, Nan Y, et al. Guidelines for the prevention and treatment of chronic hepatitis B (version 2022). Infect Dis Immun. 2023;3:145–62.
7. Porcelli S, Yockey CE, Brenner MB, Balk SP. Pillars article: analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- α/β T cells demonstrates preferential use of several Vβ genes and an invariant TCR α chain. J Exp Med. 1993;178:1–16.
8. Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: New guardians of the liver. Clin Transl Immunology. 2016;5:e98.
9. Corbett AJ, Eckle SBG, Birkinshaw RW, Liu L, Patel O, Mahony J, et al. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. Nature. 2014;509:361–5.
10. McWilliam HEG, Villadangos JA. MR1 antigen presentation to MAIT cells and other MR1-restricted T cells. Nat Rev Immunol. 2024;24:178–92.
11. Ussher JE, Bilton M, Attwod E, Shadwell J, Richardson R, de Lara C, et al. CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. Eur J Immunol. 2014;44:195–203.
12. Jeffery HC, van Wilgenburg B, Kurioka A, Parekh K, Stirling K, Roberts S, et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. J Hepatol. 2016;64:1118–27.
13. Hinks TSC, Marchi E, Jabeen M, Olshansky M, Kurioka A, Pediongco TJ, et al. Activation and in vivo evolution of the MAIT cell transcriptome in mice and humans reveals tissue repair functionality. Cell Rep. 2019;28:3249–262.e5.
14. Riva A, Patel V, Kurioka A, Jeffery HC, Wright G, Tarff S, et al. Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. Gut. 2018;67:918–30.
15. Bolte FJ, O'Keefe AC, Webb LM, Serti E, Rivera E, Liang TJ, et al. Intra-hepatic depletion of mucosal-associated invariant T cells in hepatitis C virus-induced liver inflammation. Gastroenterology. 2017;153:1392–1403.e2.
16. Liu Y, Zhu P, Wang W, Tan X, Liu C, Chen Y, et al. Mucosal-associated invariant T cell dysregulation correlates with conjugated bilirubin level in chronic HBV infection. Hepatology. 2021;73:1671–87.
17. Yan J, Allen S, McDonald E, Das I, Mak JYW, Liu L, et al. MAIT cells promote tumor initiation, growth, and metastases via tumor MR1. Cancer Discov. 2020;10:124–41.
18. Wang K, Sun Q, Zhong X, Zeng M, Zeng H, Shi X, et al. Structural mechanism for GSDMD targeting by autoprocessed caspases in pyroptosis. Cell. 2020;180:941–955.e20.
19. Newton K, Strasser A, Kayagaki N, Dixit VM. Cell death. Cell. 2024;187:235–56.
20. Lan P, Fan Y, Zhao Y, Lou X, Monsour HP, Zhang X, et al. TNF superfamily receptor OX40 triggers invariant NKT cell pyroptosis and liver injury. J Clin Invest. 2017;127:2222–34.
21. Xia P, Xing XD, Yang CX, Liao XJ, Liu FH, Huang HH, et al. Activation-induced pyroptosis contributes to the loss of MAIT cells in chronic HIV-1 infected patients. Mil Med Res. 2022;9:24.
22. Zhang LP, Wang HF, Zhai XR, Zhou CB, Yuan JH, Ma YN, et al. Pyroptotic MAITs link microbial translocation with severity of alcohol-associated liver disease. Hepatol Commun. 2023;7:e0159.
23. Zheng D, Liu J, Piao H, Zhu Z, Wei R, Liu K. ROS-triggered endothelial cell death mechanisms: Focus on pyroptosis, parthanatos, and ferroptosis. Front Immunol. 2022;13:1039241.
24. Wang J, Wu Z, Zhu M, Zhao Y, Xie J. ROS induced pyroptosis in inflammatory disease and cancer. Front Immunol. 2024;15:1378990.
25. Ginès P, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. Lancet. 2021;398:1359–76.
26. Bedner E, Smolewski P, Amstad P, Darzynkiewicz Z. Activation of caspases measured in situ by binding of fluorochrome-labeled inhibitors of caspases (FLICA): Correlation with DNA fragmentation. Exp Cell Res. 2000;259:308–13.
27. Lu W, Demers AJ, Ma F, Kang G, Yuan Z, Wan Y, et al. Next-generation mRNA sequencing reveals pyroptosis-induced CD4+ T Cell Death in Early Simian Immunodeficiency Virus-Infected Lymphoid Tissues. J Virol. 2016;90:1080–7.
28. Zhang Q, He Y, Luo N, Patel SJ, Han Y, Gao R, et al. Landscape and dynamics of single immune cells in hepatocellular carcinoma. Cell. 2019;179:829–845.e20.
29. Zhang C, Li J, Cheng Y, Meng F, Song JW, Fan X, et al. Single-cell RNA sequencing reveals intrahepatic and peripheral immune characteristics related to disease phases in HBV-infected patients. Gut. 2023;72:153–67.
30. Bai Q, Hong X, Lin H, He X, Li R, Hassan M, et al. Single-cell landscape of immune cells in human livers affected by HBV-related cirrhosis. JHEP Rep. 2023;5:100883.
31. Mohs A, Otto T, Schneider KM, Peltzer M, Boekschoten M, Holland CH, et al. Hepatocyte-specific NRF2 activation controls fibrogenesis and carcinogenesis in steatohepatitis. J Hepatol. 2021;74:638–48.
32. Li J, Lou J, Yu G, Chen Y, Chen R, Chen Z, et al. Targeting TFE3 protects against lysosomal malfunction-induced pyroptosis in random skin flaps via ROS elimination. Front Cell Dev Biol. 2021;9:643996.
33. Legoux F, Salou M, Lantz O. MAIT cell development and functions: The microbial connection. Immunity. 2020;53:710–23.
34. Bertrand L, Lehuen A. MAIT cells in metabolic diseases. Mol Metab. 2019;27s(suppl):S114–s121.
35. Liu Y, Pan R, Ouyang Y, Gu W, Xiao T, Yang H, et al. Pyroptosis in health and disease: Mechanisms, regulation and clinical perspective. Signal Transduct Target Ther. 2024;9:245.
36. Yang S, Lian G. ROS and diseases: Role in metabolism and energy supply. Mol Cell Biochem. 2020;467:1–12.

37. Ushio-Fukai M, Ash D, Nagarkoti S, Belin de Chantemèle EJ, Fulton DJR, Fukai T. Interplay between reactive oxygen/reactive nitrogen species and metabolism in vascular biology and disease. Antioxid Redox Signal. 2021;34:1319–54.
38. Fairley LH, Das S, Dharwal V, Amorim N, Hegarty KJ, Wadhwa R, et al. Mitochondria-targeted antioxidants as a therapeutic strategy for chronic obstructive pulmonary disease. Antioxidants (Basel). 2023;12:973.
39. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. Cell. 2017;171:273–85.
40. Swanson KV, Deng M, Ting JPY. The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. Nat Rev Immunol. 2019;19:477–89.
41. Jiang W, Huang Y, Han N, He F, Li M, Bian Z, et al. Quercetin suppresses NLRP3 inflammasome activation and attenuates histopathology in a rat model of spinal cord injury. Spinal Cord. 2016;54:592–6.
42. Halliwell B. Understanding mechanisms of antioxidant action in health and disease. Nat Rev Mol Cell Biol. 2024;25:13–33.
43. Zhang TT, Hu T, Jiang JG, Zhao JW, Zhu W. Antioxidant and anti-inflammatory effects of polyphenols extracted from Ilex latifolia Thunb. RSC Adv. 2018;8:7134–41.
44. Mantovani G, Macciò A, Madeddu C, Mura L, Massa E, Gramignano G, et al. Reactive oxygen species, antioxidant mechanisms and serum cytokine levels in cancer patients: Impact of an antioxidant treatment. J Cell Mol Med. 2002;6:570–82.
45. Hagel JP, Garner LC, Bilton M, Mehta H, Leng T, Hackstein CP, et al. Human MAIT cell activation in vitro. Methods Mol Biol. 2020;2098:97–124.
46. Garner LC, Amini A, FitzPatrick MEB, Lett MJ, Hess GF, Filipowicz Sinnreich M, et al. Single-cell analysis of human MAIT cell transcriptional, functional and clonal diversity. Nat Immunol. 2023;24:1565–78.
47. Dias J, Hengst J, Parrot T, Leeansyah E, Lunemann S, Malone DFG, et al. Chronic hepatitis delta virus infection leads to functional impairment and severe loss of MAIT cells. J Hepatol. 2019;71:301–12.
48. Kartasheva-Ebertz D, Gaston J, Lair-Mehiri L, Mottez E, Buivan TP, Massault PP, et al. IL-17A in human liver: Significant source of inflammation and trigger of liver fibrosis initiation. Int J Mol Sci. 2022;23:9773.
49. Nel I, Beaudoin L, Gouda Z, Rousseau C, Soulard P, Rouland M, et al. MAIT cell alterations in adults with recent-onset and long-term type 1 diabetes. Diabetologia. 2021;64:2306–21.
50. Hegde P, Weiss E, Paradis V, Wan J, Mabire M, Sukriti S, et al. Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver. Nat Commun. 2018;9:2146.

How to cite this article: Xu Z, Xu Z, Fan X, Qu MM, Wang H, Li J, et al. Antioxidant interventions reduced cytokine-induced pyroptosis of peripheral MAIT cells in patients with HBV-related cirrhosis. Hepatol Commun. 2025;9:e0714. https://doi.org/10.1097/HC9.0000000000000714