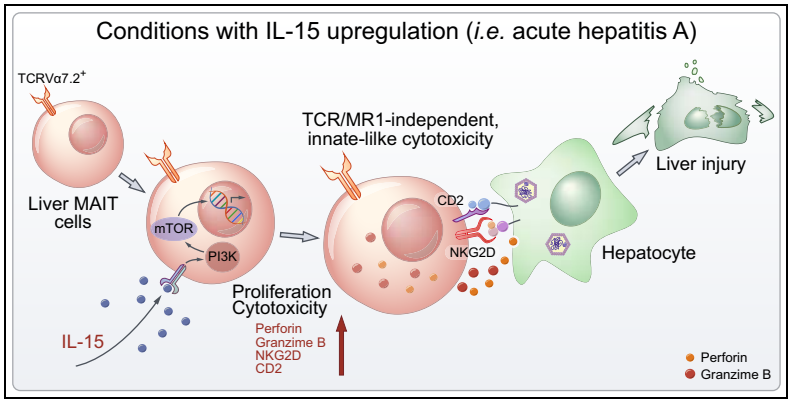
Research Article
Experimental and Translational Hepatology

JOURNAL
OF HEPATOLOGY

Human liver CD8$^+$ MAIT cells exert TCR/MR1-independent innate-like cytotoxicity in response to IL-15

Graphical abstract



Highlights

- MAIT cells activated by IL-15 exert TCR/MR1-independent, innate-like cytotoxicity.
- Innate-like cytotoxicity of MAIT cells is dependent on NKG2D, granzyme B, and CD2.
- PI3K–mTOR signaling is required for innate-like cytotoxicity of MAIT cells.
- MAIT cells exhibit activated and cytotoxic phenotypes during acute hepatitis A.
- MAIT cells may contribute to liver injury during acute hepatitis A.

Authors

Min-Seok Rha, Ji Won Han, Jong Hoon Kim, …, Dong Jin Joo, Su-Hyung Park, Eui-Cheol Shin

Correspondence

ecshin@kaist.ac.kr (E.-C. Shin), park3@kaist.ac.kr (S.-H. Park), djjoo@yuhs.ac (D.J. Joo).

Lay summary

Immune-mediated liver injury commonly occurs during viral infections of the liver. Mucosal-associated invariant T (MAIT) cells are the most abundant innate-like T cells in the human liver. Herein, we have identified a mechanism by which MAIT cells circumvent conventional T cell receptor interactions to exert cytotoxicity. We show that this innate-like cytotoxicity is increased during acute hepatitis A virus infection and correlates with the degree of hepatocyte injury.

https://doi.org/10.1016/j.jhep.2020.03.033

Research Article
Experimental and Translational Hepatology

JOURNAL OF HEPATOLOGY

Human liver CD8$^+$ MAIT cells exert TCR/MR1-independent innate-like cytotoxicity in response to IL-15

Min-Seok Rha[1], Ji Won Han[1,2], Jong Hoon Kim[1,3], June-Young Koh[1], Hye Jung Park[4], Soon Il Kim[5], Myoung Soo Kim[5], Jae Geun Lee[5], Hyun Woong Lee[6], Dong Hyeon Lee[7], Won Kim[7], Jun Yong Park[4], Dong Jin Joo[5,8,*], Su-Hyung Park[1,*], Eui-Cheol Shin[1,*]

[1]*Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea;* [2]*Division of Hepatology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul St. Mary's Hospital, Seoul 06591, Republic of Korea;* [3]*Department of Dermatology and Cutaneous Biology Research Institute, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul 06273, Republic of Korea;* [4]*Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul 03722, Republic of Korea;* [5]*Department of Surgery, Severance Hospital, Yonsei University College of Medicine, Seoul 03722, Republic of Korea;* [6]*Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul 06273, Republic of Korea;* [7]*Department of Internal Medicine, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul 07061, Republic of Korea;* [8]*The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul 03722, Republic of Korea*

Background & Aims: Mucosal-associated invariant T (MAIT) cells, the most abundant innate-like T cells in the human liver, can be activated by cytokines during viral infection without TCR stimulation. Here, we examined the mechanisms underlying TCR/MR1-independent innate-like cytotoxicity of cytokine-activated liver MAIT cells. We also examined the phenotype and function of MAIT cells from patients with acute viral hepatitis.

Methods: We obtained liver sinusoidal mononuclear cells from donor liver perfusate during liver transplantation and examined the effect of various cytokines on liver MAIT cells using flow cytometry and *in vitro* cytotoxicity assays. We also obtained peripheral blood and liver-infiltrating T cells from patients with acute hepatitis A (AHA) and examined the phenotype and function of MAIT cells using flow cytometry.

Results: IL-15-stimulated MAIT cells exerted granzyme B-dependent innate-like cytotoxicity in the absence of TCR/MR1 interaction. PI3K–mTOR signaling, NKG2D ligation, and CD2-mediated conjugate formation were critically required for this IL-15-induced innate-like cytotoxicity. MAIT cells from patients with AHA exhibited activated and cytotoxic phenotypes with higher NKG2D expression. The innate-like cytotoxicity of MAIT cells was significantly increased in patients with AHA and correlated with serum alanine aminotransferase levels.

Conclusions: Taken together, the results demonstrate that liver MAIT cells activated by IL-15 exert NKG2D-dependent innate-like cytotoxicity in the absence of TCR/MR1 engagement. Furthermore, the innate-like cytotoxicity of MAIT cells is associated with liver injury in patients with AHA, suggesting that MAIT cells contribute to immune-mediated liver injury.

Lay summary: Immune-mediated liver injury commonly occurs during viral infections of the liver. Mucosal-associated invariant T (MAIT) cells are the most abundant innate-like T cells in the human liver. Herein, we have identified a mechanism by which MAIT cells circumvent conventional T cell receptor interactions to exert cytotoxicity. We show that this innate-like cytotoxicity is increased during acute hepatitis A virus infection and correlates with the degree of hepatocyte injury.

Introduction

The liver is a target organ for several viruses, including hepatitis viruses A to E.[1,2] The liver acts as a frontline barrier against diverse gut-derived bacteria, including both commensal and pathogenic bacteria.[3,4] The liver is also a unique organ in terms of immune cell composition. In particular, innate-like T cells are enriched in the liver microenvironment compared to peripheral blood (PB) and other organs.[5,6] Innate-like T cells carrying less variant T cell receptor (TCR) can exert innate-like effector functions with specificity for commonly shared antigens among multiple pathogens, whereas conventional T cells carrying variant αβTCR exert antigen-specific adaptive responses.[7,8] Innate-like T cells that contribute to rapid immune responses include natural killer T (NKT) cells, γδ T cells, and mucosal-associated invariant T (MAIT) cells.[8]

MAIT cells are characterized by a semi-invariant TCR composed of Vα7.2-Jα33/12/20 and a restricted set of β chains in humans.[9,10] MAIT cells are present in the PB, mucosal tissues, and the liver, and are particularly localized in the liver sinusoidal space, comprising 10–40% of intrahepatic T cells.[11–13] The TCRs of

Keywords: Mucosal-associated invariant T cells; IL-15; Cytotoxicity; Hepatitis; Virus.
Received 23 October 2019; received in revised form 26 February 2020; accepted 23 March 2020; available online 2 April 2020

* Corresponding authors. Addresses: Laboratory of Immunology and Infectious Diseases, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea. Tel.: +82-42-350-4236, fax: +82-42-350-4240 (E.-C. Shin), or Laboratory of Translational Immunology and Vaccinology, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea. Tel.: +82-42-350-4248, fax: +82-42-350-4240 (S.-H. Park), or Department of Surgery, Severance Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea. Tel.: +82-2-2228-2131, fax: +82-2-313-8289 (D.J. Joo).
E-mail addresses: ecshin@kaist.ac.kr (E.-C. Shin), park3@kaist.ac.kr (S.-H. Park), djjoo@yuhs.ac (D.J. Joo).
https://doi.org/10.1016/j.jhep.2020.03.033

MAIT cells recognize microbial-derived riboflavin (vitamin B2) biosynthesis intermediates, such as 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), presented by the highly conserved major histocompatibility complex (MHC) class I-related molecule 1 (MR1).[14,15] Consequently, MAIT cells are typically activated during bacterial or fungal infections[16] and produce pro-inflammatory cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF), and interleukin (IL)-17.[11] In addition, MAIT cells can exert cytolytic activity against cells presenting a bacterial ligand on MR1.[17,18]

MAIT cells can also be activated in the absence of TCR/MR1 signaling.[19–22] For example, MAIT cells can be activated by IL-12 and IL-18, which are produced by monocytes stimulated by diverse pathogen-associated molecular patterns, to produce IFN-γ and TNF.[22,23] IL-12 and IL-18 also play a role in the TCR-independent activation of MAIT cells during infection by viruses, including hepatitis C virus,[19] dengue virus,[24] and influenza virus.[21] Although previous studies on cytokine-induced activation of MAIT cells have focused primarily on the role of IL-12 and IL-18, other cytokines can activate MAIT cells. For example, IL-15 induces IFN-γ production by MAIT cells when combined with IL-12 or IL-18.[19] Though the TCR/MR1-independent, cytokine-induced activation of MAIT cells has been widely studied, whether MAIT cells exert cytokine-induced cytolytic activity in the absence of TCR/MR1 interaction remains unknown. Which cytokines stimulate the cytolytic activity of MAIT cells also remains to be elucidated.

Recently, a critical role of TCR-independent activation of $CD8^+$ αβ T cells with other antigen specificities was reported in immune-mediated liver injury during acute viral hepatitis. IL-15, which is produced in the virus-infected liver, activates memory $CD8^+$ αβ T cells without cognate antigen stimulation during acute hepatitis A (AHA).[25] $CD8^+$ T cells activated by IL-15 exert TCR-independent innate-like cytotoxicity, resulting in liver injury during AHA.[25] However, the phenotype and function of MAIT cells that are enriched in the liver during AHA were not investigated in the previous study, particularly the TCR-independent innate-like cytotoxicity of MAIT cells.

In the present study, we aimed to examine whether liver MAIT cells stimulated by cytokines can exert TCR/MR1-independent innate-like cytotoxicity, while also assessing the mechanisms that underlie this process. We also examined the phenotype and function of MAIT cells in patients with AHA, looking at their possible contribution to liver injury.

Materials and methods

Study samples and lymphocyte isolation

Paired PB and liver perfusates were obtained from 106 healthy living liver transplant donors who were negative for HBV DNA, HCV RNA, anti-HIV antibody, and HIV p24 antigen. Graft livers were perfused with Custodiol® HTK (Essential Pharmaceuticals) solution during the bench procedure. Of the 1,000 ml of total perfusate, the first 500 ml was discarded and the second 500 ml collected and filtered. PB mononuclear cells (PBMCs) and liver sinusoidal mononuclear cells (LSMCs) were isolated by density gradient centrifugation using Lymphocyte separation medium (Corning). In addition, PB samples were obtained from 14 patients with AHA and 8 patients with chronic hepatitis B (CHB). Characteristics of the patients are presented in Table S1–3. To analyze liver tissue-infiltrating lymphocytes, background non-tumor liver tissues were obtained from 6 patients with colon cancer liver metastasis during tumor resection. Liver tissues were also obtained from 4 patients with AHA. For flow cytometric analyses of tissue-infiltrating lymphocytes, single-cell suspensions were prepared using a Tumor Dissociation Kit (Miltenyi Biotec) in combination with a gentleMACS™ dissociator (Miltenyi Biotec). After isolation, cells were either cryopreserved in FBS (RMBIO) with 10% DMSO (Sigma-Aldrich) or immediately used for experiments. This study was reviewed and approved by the institutional review board of Severance Hospital (Seoul, Republic of Korea; 2013-1071-001 and 4-2016-0406) and conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all study participants.

Multicolor flow cytometry

Cryopreserved PBMCs and LSMCs were thawed and stained with fluorochrome-conjugated antibodies for specific surface markers for 10 min at room temperature. Dead cells were excluded using LIVE/DEAD red fluorescent reactive dye or near-infrared fluorescent reactive dye (Invitrogen). For tetramer staining, the cells were stained with tetramers for 20 min at room temperature, washed twice, and then stained using the protocols as described above. To stain intracellular markers, cells were fixed and permeabilized using a FoxP3 staining buffer kit (eBioscience) and then stained with intracellular markers for 30 min at 4°C. Multicolor flow cytometry was performed using an LSR II instrument (BD Biosciences) and data analyzed using FlowJo software (FlowJo, LLC). Fluorochrome-conjugated monoclonal antibodies used in this study are listed in the CTAT table. Biotinylated human MR1 5-OP-RU tetramers were provided by the NIH Tetramer Core Facility and prepared using streptavidin-PE (Invitrogen).

For further details regarding the materials and methods used, please refer to the supplementary information and CTAT table.

Results

Liver MAIT cells exhibit activation and NK-like phenotypes

To investigate the characteristics of liver MAIT cells, we analyzed paired PBMCs and LSMCs from heathy donors (HDs). MAIT cells were defined as $CD3^+CD8^+CD4^-TCRV\alpha7.2^+CD161^{hi}$ cells (Fig. S1A) and confirmed by MR1 5-OP-RU tetramer staining (Fig. S1B and C).[26] As described previously,[12] the frequency of MAIT cells among $CD3^+$ T cells was higher in liver sinusoids than PB (Fig. 1A), and the frequency of MAIT cells among PB $CD3^+$ T cells was positively correlated with that among liver $CD3^+$ T cells (Fig. 1B). In the TCRVβ repertoire analysis, TCRVβ usage was almost identical between the 2 compartments (Fig. 1C). The expression level of T cell-related transcription factors was also similar between the 2 populations (Fig. 1D). However, higher frequencies of liver MAIT cells were $CD38^+$, PD-1^+, $CD69^+$, $CD56^+$, and $NKG2D^+$ compared to PB MAIT cells (Fig. 1E), indicating that liver MAIT cells are generally more activated and exhibit phenotypes that are associated with NK cells and tissue-residency.

IL-15 strongly induces MAIT cells to proliferate and upregulate cytotoxic molecules

Next, we analyzed the proliferation and effector functions of liver MAIT cells exposed to various cytokines. IL-2, IL-7, and IL-15, but not IL-12 and IL-18, significantly induced the proliferation of liver MAIT cells in CellTrace Violet dilution assays (Fig. 2A and B). This finding was corroborated by an analysis of Ki-67 expression (Fig. 2C and D). When cytokine production was investigated, the production of IFN-γ and TNF by liver MAIT cells was significantly increased by IL-12 and IL-15, but not by IL-2, IL-7, or IL-18 (Fig. 2E). IL-17A

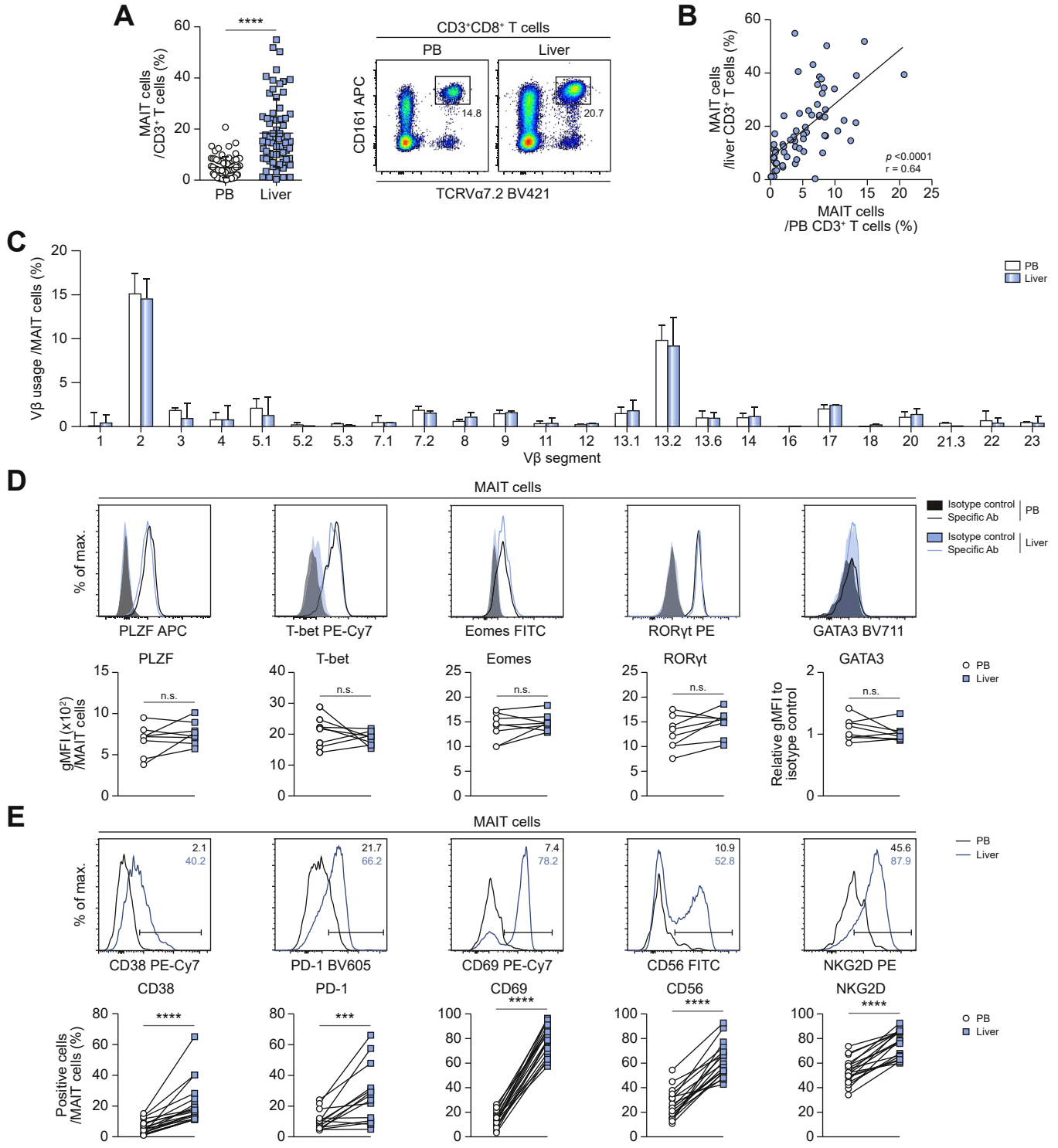


Fig. 1. Phenotypic characterization of liver MAIT cells. Paired PBMCs and LSMCs from HDs were analyzed by flow cytometry. (A) The frequency of TCRVα7.2^{+}CD161^{hi}CD8^{+} MAIT cells among PB and liver sinusoidal CD3^{+} T cells was examined (n = 68). The right panel shows representative dot plots from a single donor. Bars and error bars represent mean and standard deviation (SD). (B) The frequency of MAIT cells among PB CD3^{+} T cells was plotted against that among liver sinusoidal CD3^{+} T cells (n = 68). Bars graphs represent median and range. (C) The percentage of PB (white) and liver (blue) MAIT cells expressing each Vβ chain was analyzed by flow cytometry (n = 3). (D) The expression level of PLZF, T-bet, Eomes, GATA3, and RORγt was analyzed in PB (black) and liver (blue) MAIT cells (n = 8). Representative flow cytometry plots (upper) and summary data (lower) are presented. (E) The percentages of CD38^{+} (n = 18), PD-1^{+} (n = 14), CD69^{+} (n = 18), CD56^{+} (n = 18), and NKG2D^{+} (n = 18) cells were analyzed in PB and liver MAIT cells. Representative flow cytometry plots (upper) and summary data (lower) are presented. Statistical analysis was performed using the paired *t* test (A), the parametric Pearson correlation test (B), or Wilcoxon signed-rank test (D and E). n.s., not significant, ***p <0.001, ****p <0.0001. HDs, heathy donors; LSMCs, liver sinusoidal mononuclear cells; MAIT, mucosal-associated invariant T; MFI, mean fluorescence intensity; PB, peripheral blood; PBMCs, PB mononuclear cells.

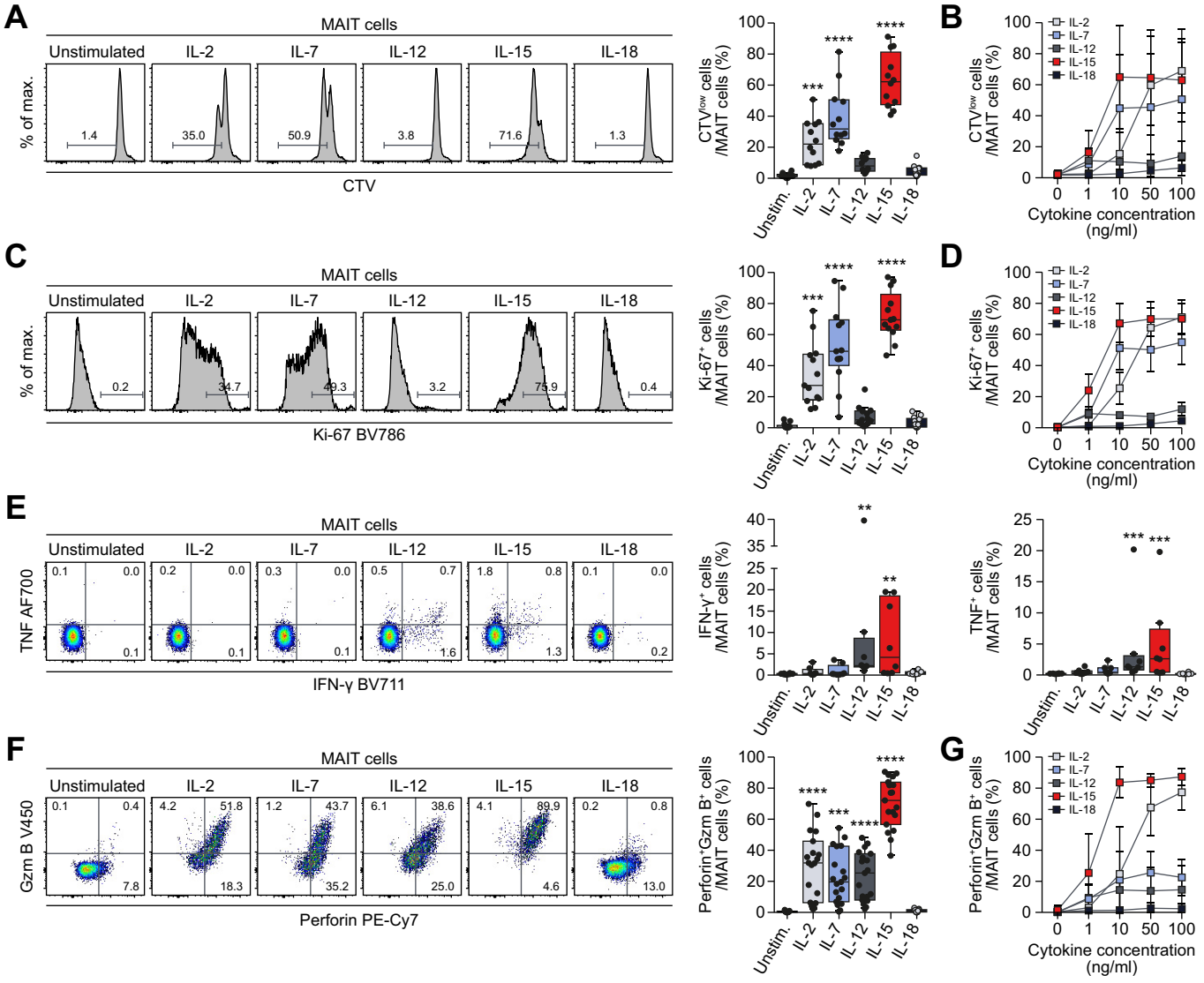


Fig. 2. Proliferation and effector functions of liver MAIT cells following cytokine stimulation. CellTrace Violet-labeled (A and B) or non-labeled (C–G) LSMCs from HDs were incubated with IL-2, IL-7, IL-12, IL-15, or IL-18 and analyzed by flow cytometry. Cytokines were used at fixed doses: IL-2 (10 ng/ml), IL-7 (10 ng/ml), IL-12 (50 ng/ml), IL-15 (10 ng/ml), or IL-18 (50 ng/ml) (A, C, E, F) or at indicated doses (B, D, G). (A and B) Following cytokine stimulation, LSMCs were incubated for 96 h and analyzed for the percentage of CellTrace Violet^{low} cells among MAIT cells (A, n = 12; B, n = 6). The left panel shows representative data from a single donor. (C and D) Following cytokine stimulation, LSMCs were incubated for 96 h and analyzed for the percentage of Ki-67$^+$ cells among MAIT cells (C, n = 13; D, n = 6). The left panel shows representative data from a single donor. (E) Following cytokine stimulation, LSMCs were incubated for 24 h, and intracellular cytokine staining was performed to examine the percentage of IFN-γ^+ and TNF$^+$ cells among the MAIT cells (n = 8). The left panel shows representative dot plots from a single donor. (F and G) Following cytokine stimulation, LSMCs were incubated for 48 h and analyzed for the percentage of perforin$^+$granzyme B$^+$ cells among MAIT cells (F, n = 19; G, n = 6). The left panel shows representative flow cytometry plots from a single donor. Box plots represent the IQR, with the horizontal line indicating the median. Whiskers extend to the farthest data point within a maximum of 1.5× IQR. The Friedman test with Dunns' multiple comparisons test was used to determine differences between cytokine-stimulated and unstimulated cells (A, C, E, F). $^{**}p$ <0.01, $^{***}p$ <0.001, $^{****}p$ <0.0001. Gzm, granzyme; HDs, heathy donors; IFN, interferon; IL, interleukin; LSMCs, liver sinusoidal mononuclear cells; MAIT, mucosal-associated invariant T; TNF, tumor necrosis factor.

production was not increased by any cytokine (Fig. S2). We also examined the expression of cytotoxic molecules in liver MAIT cells after cytokine stimulation. IL-2, IL-7, IL-12, and IL-15 each significantly increased the percentage of perforin$^+$granzyme B$^+$ MAIT cells (Fig. 2F and G). The effects of cytokines on MAIT cell proliferation and cytotoxicity were confirmed using magnetically sorted liver MAIT cells (Fig. S3A–E). IL-15-induced proliferation and upregulation of perforin and granzyme B were also observed in PB MAIT cells (Fig. S4A–C). Taken together, these data indicate that IL-15 plays an important role in activating MAIT cells, resulting in proliferation and upregulation of cytotoxic molecules.

IL-15-stimulated liver MAIT cells kill target cells in an NKG2D-dependent manner in the absence of TCR/MR1 interactions

As perforin and granzyme B were upregulated in liver MAIT cells following cytokine treatment, we next evaluated whether these cells could mediate TCR/MR1-independent, innate-like killing of target cells. MAIT cells were magnetically sorted from LSMCs, stimulated with various cytokines, and co-cultured with K562 cells, which do not express surface MR1 (Fig. S5A).[27] The absence of functioning MR1 on the cell surface of K562 was confirmed by the fact that MAIT cells were not activated at all by *E. coli*-treated K562 cells whereas they were activated by *E. coli*-treated THP-1

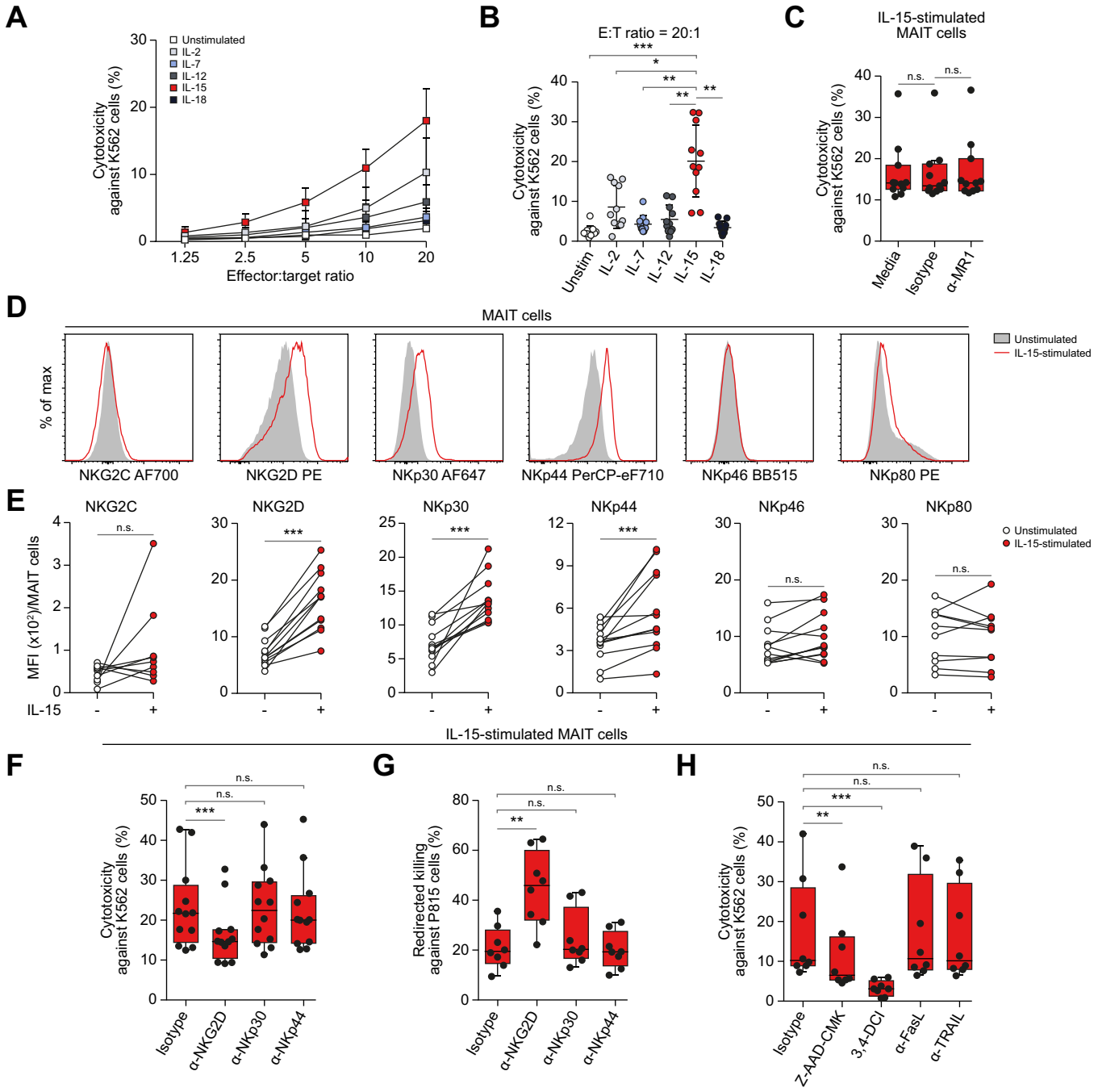


Fig. 3. NKG2D- and granzyme B-dependent innate-like cytotoxicity of IL-15-stimulated liver MAIT cells. (A and B) Magnetically sorted MAIT cells from LSMCs were cultured with or without each cytokine (IL-2 (10 ng/ml), IL-7 (10 ng/ml), IL-12 (50 ng/ml), IL-15 (10 ng/ml), or IL-18 (50 ng/ml)) for 48 h and then co-cultured with PKH67-labeled K562 cells at various effector:target (E:T) ratios for 12 h. Cytotoxicity was evaluated by staining with TO-PRO-3-iodide and flow cytometric analysis. Summary data for cytotoxicity against K562 cells at various E:T ratios (A, n = 8) and at 20:1 (B, n = 11) are presented. Dots and error bars represent median and IQR (A). Horizontal lines and error bars represent mean and SD (B). (C) The cytotoxicity of IL-15-stimulated MAIT cells was evaluated in the presence of indicated antibodies (n = 11) (D and E) LSMCs were cultured with or without IL-15 (10 ng/ml) for 48 h. The MFI of NKG2C (n = 9), NKG2D (n = 12), NKp30 (n = 12), NKp44 (n = 12), NKp46 (n = 12), and NKp80 (n = 10) was analyzed in MAIT cells in the presence or absence of IL-15 stimulation. Representative histograms (D) and summary data (E) are shown. (F–H) The cytotoxicity of IL-15-stimulated MAIT cells against K562 cells (F and H) or P815 cells (G) was evaluated in the presence of the indicated antibodies (10 μg/ml) or inhibitors (100 μM) (F, n = 12; G, n = 8; and H, n = 8). The E:T ratio in the K562 killing assay was 20:1, and the E:T ratio in the P815-redirected killing assay was 10:1. Box plots represent the IQR, with the horizontal line indicating the median. Whiskers extend to the farthest data point within a maximum of 1.5× IQR. Statistical analysis was performed using one-way repeated-measures ANOVA with Tukey's multiple comparisons test (B), the Friedman test with Dunns' multiple comparisons test (C, F–H), or the Wilcoxon signed-rank test (E). n.s., not significant, $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001. IL, interleukin; LSMCs, liver sinusoidal mononuclear cells; MAIT, mucosal-associated invariant T; MFI, mean fluorescence intensity.

cells (Fig. S5B). As shown in Fig. 3A and B, IL-15 predominantly enhanced K562 killing by MAIT cells compared to other cytokines, though NK cells exhibited stronger IL-15-induced cytotoxicity against K562 cells (Fig. S6). Anti-MR1 blocking antibody did not abrogate the K562 cytotoxicity of IL-15-stimulated liver MAIT cells, confirming that this cytotoxicity was MR1 independent (Fig. 3C). We also confirmed that magnetic sorting of MAIT cells using anti-TCRVα7.2 antibodies did not activate the cells (Fig. S7). IL-15-induced cytotoxicity was also observed when MAIT cells were sorted for either MR1 5-OP-RU tetramer$^+$ cells or TCRVα7.2$^+$CD161^{hi} cells (Fig. S8A–D). This IL-15-induced cytotoxicity was more exaggerated in liver MAIT cells than in PB MAIT cells (Fig. S9). In addition, IL-15-stimulated liver MAIT cells readily killed liver-derived Huh-7 cells (Fig. S10).

We hypothesized that NK-activating receptors are responsible for the TCR/MR1-independent, innate-like cytotoxicity of IL-15-stimulated MAIT cells. To test this hypothesis, we examined MAIT cells for expression of several NK-activating receptors, including NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80, and DNAM-1. The expression of NKG2D, NKp30, NKp44, and DNAM-1 was significantly increased by IL-15, suggesting that these receptors may be involved in the induction of innate-like cytotoxicity (Fig. 3D, E, and S11A). To formally determine which receptors mediate the innate-like cytolytic activity of IL-15-stimulated MAIT cells, we used receptor blocking antibodies. As shown in Fig. 3F and S11B, cytotoxicity against K562 was significantly blocked by anti-NKG2D antibodies, but not by antibodies against other NK receptors. We observed the same results in cytotoxicity assays against Huh-7 cells (Fig. S12). The crucial role of NKG2D in the innate-like cytotoxicity of MAIT cells was confirmed using antibody-mediated redirected killing assays against P815 cells (Fig. 3G). The NKG2D expression induced by IL-15 was significantly greater in liver MAIT cells than in PB MAIT cells (Fig. S13).

We investigated the surface expression of ligands for various NK receptors on K562 and Huh-7 cells. K562 cells expressed ligands for NKG2D (MIC-A/B, ULBP-1, ULBP-2/5/6, ULBP-3, ULBP-4), NKp30 (B7-H6), and DNAM-1 (PVR, Nectin-2), but not for NKp44 (Fig. S14A). Huh-7 cells expressed some ligands for NKG2D (ULBP-1, ULBP-2/5/6, ULBP-3, ULBP-4) and DNAM-1 (PVR, Nectin-2), but not for NKp30 and NKp44 (Fig. S14B). Therefore, the NKG2D-dependent cytotoxicity of IL-15-stimulated MAIT cells is not explained by the expression pattern of ligands for NK-activating receptors on target cells.

We also investigated the roles of granzyme B, Fas ligand (FasL), and TRAIL in TCR/MR1-independent, innate-like cytotoxicity of IL-15-stimulated MAIT cells. As shown in Fig. 3H, liver MAIT cell killing of K562 cells was significantly blocked by Z-AAD-CMK, a granzyme B inhibitor, and 3,4-dichloroisocoumarin, a pan-granzyme inhibitor, but not by anti-FasL or anti-TRAIL blocking antibodies. Taken together, these data demonstrate that IL-15-stimulated liver MAIT cells kill target cells by NKG2D ligation via a granzyme B-dependent mechanism in the absence of TCR/MR1 interactions.

The IL-15-induced innate-like cytotoxicity of liver MAIT cells involves CD2-mediated conjugate formation

The cytotoxicity of NK and T cells requires conjugate formation between effector and target cells for the effective delivery of cytotoxic molecules to target cells.[28] To determine whether IL-15 stimulation enhanced liver MAIT cells' adhesion to target cells, we examined the expression of CD2 and CD11a, which play crucial roles in conjugate formation.[28,29] As shown in Fig. 4A, IL-15 significantly increased the expression of CD2 and CD11a in liver MAIT cells. In K562 cytotoxicity assays, anti-CD2 antibodies, but not anti-CD11a antibodies, significantly blocked the innate-like cytotoxicity of IL-15-stimulated liver MAIT cells (Fig. 4B). Next, we assessed conjugate formation between MAIT and K562 target cells by flow cytometry (Fig. S15). IL-15 stimulation significantly enhanced conjugation of liver MAIT cells with K562 target cells (Fig. 4C) and this conjugation was significantly blocked by anti-CD2, but not by anti-CD11a antibodies (Fig. 4D). These data indicate that the IL-15-induced innate-like cytotoxicity of liver MAIT cells involves both NKG2D-mediated triggering of cytotoxicity and CD2-mediated conjugation with target cells.

The PI3K/mTOR pathway plays a critical role in the IL-15-induced innate-like cytotoxicity of MAIT cells

IL-15 binding to its receptor complex initiates signal transduction via multiple pathways, including JAK/STAT5, Ras/Raf/MEK, and PI3K/mTOR.[30] Therefore, we investigated the signaling pathways involved in IL-15-induced innate-like cytotoxicity in MAIT cells. We first confirmed that IL-15 stimulation increased the phosphorylation of signaling proteins, including STAT5, ERK, AKT, mTOR, and S6 (Fig. 5A). Next, liver MAIT cells were stimulated by IL-15 following pre-treatment with specific inhibitors for each signaling pathway: wortmannin, a PI3K inhibitor; PP242, an mTOR inhibitor; PD98059, a MEK inhibitor; and pimozide, a STAT5 inhibitor. An IL-15-induced increase in the frequency of granzyme B$^+$ cells was significantly abrogated by wortmannin and PP242, but not PD98059 and pimozide, whereas the increase in the frequency of perforin$^+$ cells was not abrogated by any inhibitor (Fig. 5B). When the IL-15-induced upregulation of NKG2D was examined, it was significantly decreased by wortmannin, PP242, and PD98059 (Fig. 5C). In addition, wortmannin and PP242 significantly abolished the innate-like cytotoxicity of IL-15-stimulated MAIT cells (Fig. 5D). These findings indicate that the PI3K/mTOR pathway plays a critical role in the IL-15-induced innate-like cytotoxicity of MAIT cells.

Innate-like cytotoxicity of MAIT cells correlates with liver injury during AHA

Finally, we examined the phenotypes of MAIT cells from patients with AHA, who underwent a self-limited course of hepatitis, and patients with CHB. An IL-15-induced, NKG2D-dependent, innate-like cytotoxicity of conventional memory CD8$^+$ T cells has been described in AHA, in which ligands for NKG2D are overexpressed in liver tissue.[25] First, we examined PB MAIT cells. The percentage of activated (CD38$^+$HLA-DR$^+$, Fig. 6A) cells was significantly increased in PB MAIT cells from AHA and CHB patients compared to HDs. In addition, the percentage of perforin$^+$granzyme B$^+$ (Fig. 6B) cells and the NKG2D expression level (Fig. 6C) were significantly higher in PB MAIT cells from patients with AHA, but not in those from patients with CHB, compared to HDs. We also observed increased expression of NKG2D when liver MAIT cells from patients with AHA were analyzed (Fig. 6D). Importantly, PB MAIT cells from patients with AHA exerted innate-like cytotoxicity against K562 cells whereas those from HDs did not (Fig. 6E). We found no significant differences in the relative frequency of MAIT cells among liver CD3$^+$ T cells between HDs and patients

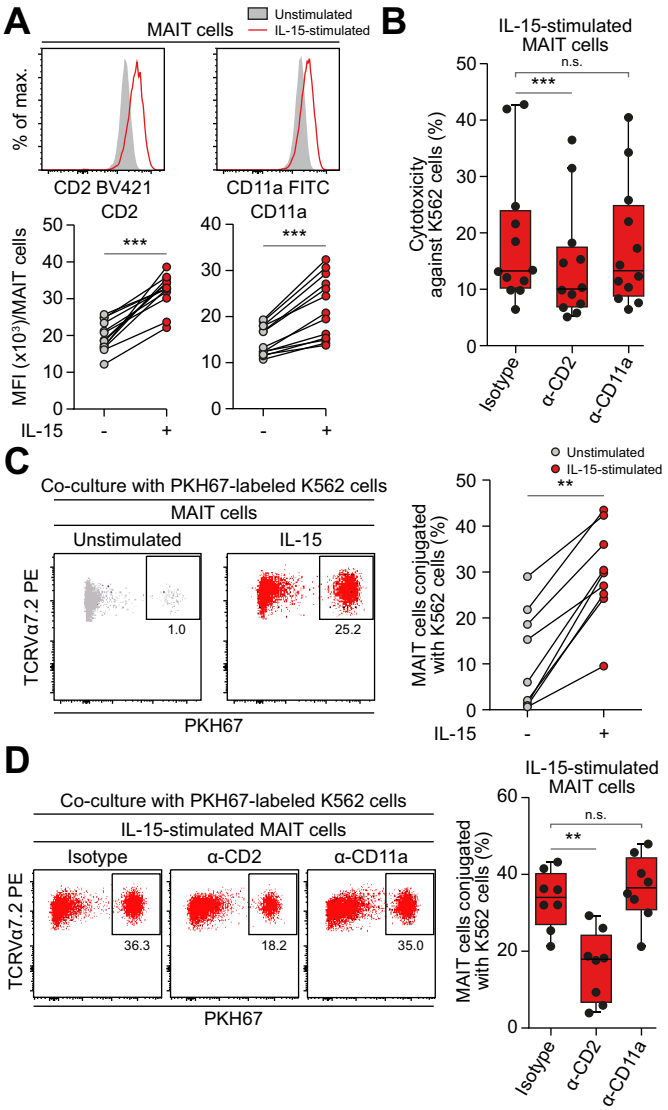


Fig. 4. CD2-mediated conjugate formation with target cells in innate-like cytotoxicity of MAIT cells. (A) LSMCs were cultured with or without IL-15 (10 ng/ml) for 48 h and expression of CD2 and CD11a analyzed in MAIT cells (n = 13). Representative flow cytometry plots (left) and summary data (right) are presented. (B) Cytotoxicity of IL-15-stimulated MAIT cells (n = 12) against K562 cells was analyzed in the presence of the indicated blocking antibodies (10 μg/ml). (C) Magnetically sorted MAIT cells from LSMCs (n = 9) were cultured with or without IL-15 (10 ng/ml) for 48 h and assessed for conjugate formation via co-incubation with PKH67-labeled K562 cells for 12 h at a 1:1 E:T ratio. The conjugate percentage was assessed by the frequency of MAIT cells that were also PKH67-positive. Representative flow cytometry plots (left) and summary data (right) are presented. (D) The percentage of IL-15-stimulated MAIT cells that formed conjugates with K562 cells was examined in the presence of the indicated blocking antibodies (10 μg/ml) (n = 8). Representative flow cytometry plots (left) and summary data (right) are presented. Box plots represent the IQR, with the horizontal line indicating the median. Whiskers extend to the farthest data point within a maximum of 1.5× IQR. Statistical analysis was performed using the Wilcoxon signed-rank test (A and C) or the Friedman test with Dunns' multiple comparisons test (B and D). n.s., not significant, $^{**}p$ <0.01, $^{***}p$ <0.001. IL, interleukin; LSMCs, liver sinusoidal mononuclear cells; MAIT, mucosal-associated invariant T; MFI, mean fluorescence intensity.

with AHA, though the relative frequency of MAIT cells among PB CD3$^+$ T cells was lower in patients with AHA than HDs (Fig. S16A and B).

We next analyzed the relationship between the phenotype and function of MAIT cells and the degree of hepatocyte injury among patients with AHA. The percentages of activated (CD38$^+$HLA-DR$^+$) and perforin$^+$granzyme B$^+$ cells in PB MAIT cells were both significantly correlated with serum alanine aminotransferase (ALT) levels, a marker of liver damage (Fig. 6F). The level of NKG2D expression of PB MAIT cells was also significantly correlated with serum ALT levels whereas that of liver MAIT cells had a tendency without significance (Fig. 6F and G). Furthermore, the innate-like cytolytic activity of PB MAIT cells from patients with AHA significantly correlated with serum ALT levels (Fig. 6H).

Furthermore, we investigated a relationship between MAIT cell activation and the clinical course of AHA by examining the phenotype of MAIT cells from the time of admission (acute stage) to the convalescent stage. The percentage of activated (CD38$^+$HLA-DR$^+$) MAIT cells and expression of cytotoxic molecules in MAIT cells were significantly decreased parallel with a decrease in serum ALT levels, and the expression of NKG2D significantly decreased in MAIT cells (Fig. 6I). These findings suggest that MAIT cells exhibit activated and cytotoxic phenotypes in the acute phase of AHA when liver injury occurs, but these phenotypes gradually subside in the convalescent phase when liver injury is resolved.

Altogether, these findings indicate that MAIT cells are activated and exert increased levels of innate-like cytotoxicity during AHA, strongly suggesting that they play a major role in immunopathological liver injury.

Discussion

Although MAIT cells have been shown to exert cytotoxic activity, previous studies have focused only on their TCR-dependent killing activity against cells presenting a bacterial ligand complexed with MR1.[17,18] Here, we showed that MAIT cells can express a TCR/MR1-independent, innate-like cytotoxic activity when stimulated by IL-15. Given that IL-15 production is frequently upregulated during viral infection, it is highly likely that MAIT cells will upregulate NKG2D and exert innate-like cytotoxic activity during viral infections.

A previous study reported that IL-15 indirectly activates MAIT cells to produce perforin, granzyme B, and IFN-γ only in the presence of monocytes that produce IL-18 in response to IL-15 stimulation.[31] However, the direct effect of IL-15 on MAIT cells was not previously elucidated. In addition, although TCR-dependent degranulation of MAIT cells is enhanced by IL-15 in the presence of monocytes,[31] the TCR/MR1-independent, NKG2D-dependent cytotoxicity of MAIT cells was not examined previously.

In recent years, NK-activating receptor-mediated, innate-like killing activity by T cells has been an area of increasing scientific interest.[32–35] Previous studies reported that IL-15 stimulates conventional memory CD8$^+$ T cells to exhibit TCR-independent cytotoxicity in an NKG2D- or NKp30-dependent manner.[25,32,34] In addition, a recent study showed that triggering of NKG2C activates CD8$^+$ T cells to release cytotoxic granules in a TCR-independent manner.[36] Invariant NKT cells, a subset of innate-like T cells that share many characteristics with MAIT cells, also exert TCR-independent, NKG2D-dependent cytotoxicity.[37] Although a previous study reported that NKG2D can co-stimulate TCR-mediated activation of MAIT cells,[38] the role of NKG2D in MAIT cells in the absence of TCR/MR1 interaction was unclear. Here, we demonstrated that IL-15-stimulated liver MAIT

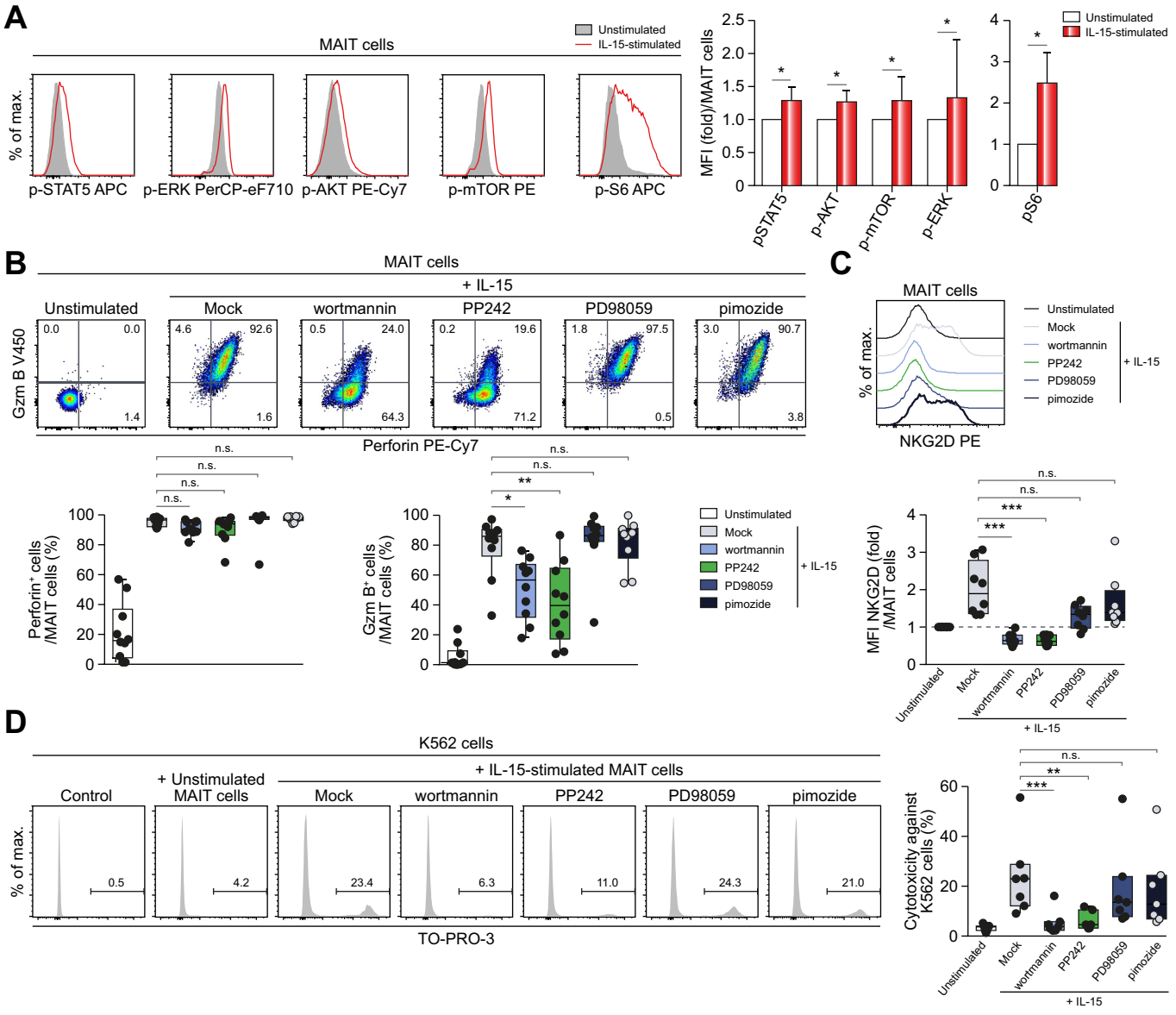


Fig. 5. Role of the PI3K/mTOR pathway in IL-15-induced innate-like cytotoxicity of liver MAIT cells. (A) LSMCs (n = 7) from HDs were stimulated with IL-15 (10 ng/ml) for 30 minutes (for p-STAT5), 1 h (for p-AKT and p-mTOR), or 2 h (for p-ERK and p-S6), and then phosphorylation of signaling proteins assessed by flow cytometry. Representative histograms (left) and the summary data (right) of the fold change relative to unstimulated cells are shown. Bar graphs represent median and IQR. (B and C) LSMCs were pre-treated with inhibitors for PI3K (wortmannin; 10 μM), mTOR (PP242; 1 μM), MEK (PD98059; 10 μM), or STAT5 (pimozide; 7.5 μM) for 1 h and then cultured with or without IL-15 (10 ng/ml) for 48 h. (B) The percentages of perforin$^+$ and granzyme B$^+$ cells among MAIT cells were analyzed (n = 10). Representative flow cytometry plots from a single donor (upper) and summary data (lower) are presented. (C) NKG2D expression was examined in MAIT cells (n = 8). Representative histograms from a single donor (upper) and summary data (lower) of the fold change relative to unstimulated cells are presented. (D) Magnetically sorted MAIT cells from LSMCs were pre-treated with wortmannin, PP242, PD98059, or pimozide for 1 h and then stimulated with IL-15 (10 ng/ml). After 48 h of IL-15 stimulation, cells were co-cultured with PKH67-labeled K562 cells at a 20:1 E:T ratio and cytotoxicity against K562 cells evaluated (n = 6). Representative histograms (left) and summary data (right) are presented. Box plots represent the IQR, with the horizontal line indicating the median. Whiskers extend to the farthest data point within a maximum of 1.5× IQR. Statistical analysis was performed using the Wilcoxon signed-rank test (A) or the Friedman test with Dunns' multiple comparisons test (B–D). n.s., not significant, $^*p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001. Gzm, granzyme; HDs, heathy donors; IL, interleukin; LSMCs, liver sinusoidal mononuclear cells; MAIT, mucosal-associated invariant T; MFI, mean fluorescence intensity.

cells also exhibit TCR-independent cytotoxicity that is mediated by NKG2D. Thus, a critical role of NKG2D in TCR-independent cytotoxic activity is observed in not only conventional CD8$^+$ T cells, but also innate-like T cells, such as NKT cells and MAIT cells. However, cytotoxicity-triggering mechanisms differ between conventional and innate-like T cells. Whereas anti-NKp30 antibodies have been reported to block the innate-like cytotoxicity of IL-15-stimulated non-MAIT CD8$^+$ T cells,[25] they did not block the cytotoxic activity of IL-15-stimulated MAIT cells in the current study.

We also demonstrated that CD2-mediated conjugate formation is an important feature of the innate-like cytotoxicity of IL-15-stimulated MAIT cells. In NK and T cells, CD2 plays a role in adhesion to target cells, as well as mediating co-stimulation

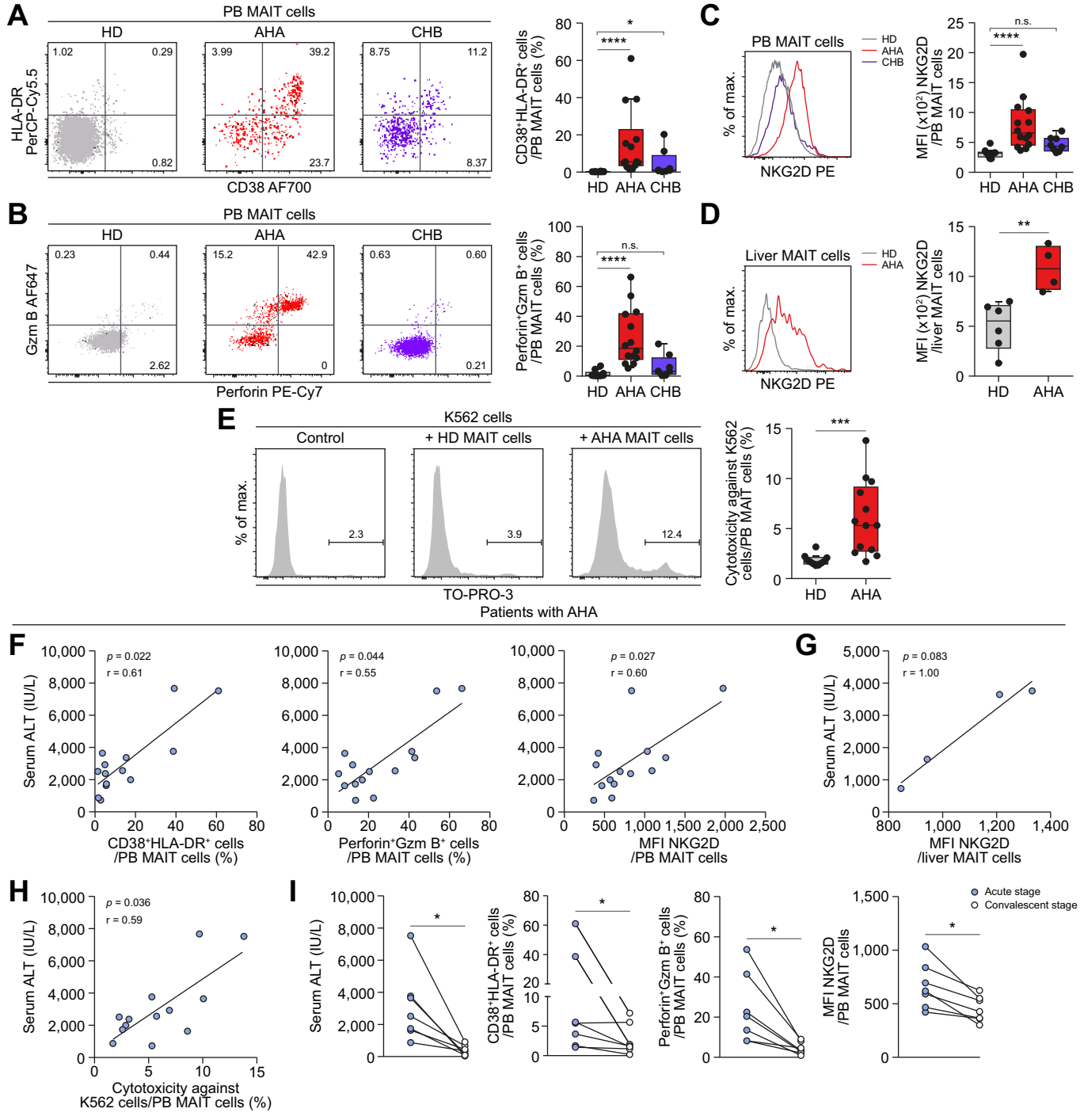


Fig. 6. Phenotypes and functions of MAIT cells from patients with viral hepatitis. (A–C) PB MAIT cells from HDs (n = 10), patients with AHA (n = 14), and patients with CHB (n = 8) were analyzed by flow cytometry. The percentage of CD38$^+$HLA-DR$^+$ (A), perforin$^+$granzyme B$^+$ (B) cells among MAIT cells and the expression level of NKG2D (C) are presented. (D) The expression level of NKG2D was examined in MAIT cells from the liver tissue of patients without viral hepatitis (n = 6) and those with AHA (n = 4). (E) Magnetically sorted MAIT cells from PBMCs of HDs (n = 10) or patients with AHA (n = 13) were co-cultured with PKH67-labeled K562 cells for 12 h at a 20:1 E:T ratio and cytotoxicity against K562 cells evaluated. Representative histograms (left) and summary data (right) are presented. (F) The percentage of CD38$^+$HLA-DR$^+$, perforin$^+$granzyme B$^+$ cells among PB MAIT cells and NKG2D expression levels on PB MAIT cells are plotted against serum ALT levels in AHA patients (n = 14). (G) NKG2D expression levels of liver MAIT cells are plotted against serum ALT levels in patients with AHA (n = 4). (H) The innate-like cytotoxicity of PB MAIT cells was plotted against serum ALT levels (n = 13). (I) Serum ALT levels, the percentages of CD38$^+$HLA-DR$^+$ and perforin$^+$granzyme B$^+$ cells among PB MAIT cells, and the NKG2D expression level in PB MAIT cells were examined in patients with AHA (n = 7) in the acute and convalescent stages. Box plots represent the IQR, with the horizontal line indicating the median. Whiskers extend to the farthest data point within a maximum of 1.5× IQR. Statistical analysis was performed using the Kruskal-Wallis test with Dunns' multiple comparisons test (A–C), the Mann-Whitney *U* test (D and E), the nonparametric Spearman's rank correlation test (F–H) or the Wilcoxon signed-rank test (I). n.s., not significant, *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001. AHA, acute hepatitis A; ALT, alanine aminotransferase; CHB, chronic hepatitis B; Gzm, granzyme; HDs, heathy donors; MAIT, mucosal-associated invariant T; MFI, mean fluorescence intensity; PB, peripheral blood; PBMCs, PB mononuclear cells.

functions.[29,39,40] Although it is well-known that lymphocyte function-associated antigen-1 (LFA-1) expressed on $CD8^+$ T cells plays a central role in TCR-mediated killing by facilitating tight adhesion to target cells,[41] the K562 cytotoxicity of IL-15-stimulated MAIT cells was significantly reduced by blocking CD2, but not by CD11a, an alpha subunit of LFA-1. These data suggest that the NKG2D-mediated immunological synapse formed between MAIT cells and their target may differ from the immunological synapse formed during TCR-mediated killing of conventional $CD8^+$ T cells.

Furthermore, we found that the downstream signaling mechanisms leading to the IL-15-induced innate-like cytotoxicity of MAIT cells mainly involve the PI3K/mTOR pathway. Although IL-15 activated not only the PI3K/mTOR pathway, but also other pathways, including the JAK/STAT5 and Ras/Raf/MEK pathways, in MAIT cells, only blockade of the PI3K/mTOR pathway significantly impaired the innate-like cytotoxicity. In previous studies, mTOR signaling was also required for the cytotoxicity of IL-15-stimulated NK cells in mice and humans.[42,43] However, the detailed mechanisms underlying IL-15-induced activation of MAIT cells may differ from those of NK cells. In contrast to the results in MAIT cells in the present study, blockade of the Ras/Raf/MEK pathway also reduced granzyme B expression in NK cells.[43] Interestingly, IL-15-induced upregulation of perforin in MAIT cells was not impaired by inhibition of the PI3K/mTOR pathway. Further studies are required to clarify the signaling pathways that regulate perforin expression in MAIT cells following IL-15 stimulation.

It is well established that MAIT cells can exert TCR-dependent cytolytic activity against cells presenting a bacterial ligand on MR1.[17,18] In addition, IL-7 enhances the TCR/MR1-dependent killing activity of MAIT cells.[44] However, the immunological significance of their cytolytic activity during infection is not well understood. Our discovery that IL-15-stimulated MAIT cells exhibit innate-like cytotoxic activity broadens the spectrum of targets killed by MAIT cells. Because the innate-like cytotoxicity depends on NKG2D rather than TCR engagement, host cells expressing NKG2D ligands can be cytotoxic targets regardless of antigen presentation by MR1. In this regard, the innate-like cytotoxicity of MAIT cells may contribute to both host immunopathology and the elimination of virus-infected cells or tumor cells that highly express NKG2D ligands.[45]

The IL-15-induced innate-like cytotoxicity of MAIT cells may be particularly important in microenvironments where NKG2D ligands are overexpressed. In the case of conventional $CD8^+$ T cells, TCR-independent, NKG2D-mediated immunopathology by bystander-activated $CD8^+$ T cells has been reported in mice[35] and humans.[32] Similarly, we previously showed that innate-like cytotoxicity of IL-15-activated bystander memory $CD8^+$ T cells is associated with liver injury during AHA.[25] Given the increased expression of cytotoxic molecules and NKG2D in MAIT cells from patients with AHA, and that MAIT cells constitute the major population of intrahepatic T cells, it is highly probable that these cells play a role in immunopathological liver injury during AHA. Indeed, the innate-like cytotoxicity of MAIT cells was significantly correlated with serum ALT levels among patients with AHA. It would be of interest to investigate the role of IL-15-activated MAIT cells in other diseases associated with dysregulated IL-15 production.

In summary, our results demonstrate that IL-15 activates liver MAIT cells to exert innate-like cytotoxicity triggered by NKG2D in the absence of TCR/MR1 engagement. These findings broaden the spectrum of cellular targets for MAIT cell cytotoxicity and suggest that MAIT cells may participate in the elimination of virus-infected cells. Moreover, our data strongly suggest that MAIT cells can play an immunopathological role in liver diseases with increased IL-15 expression, such as AHA. In this regard, MAIT cells may be regulatory targets for the management of liver diseases.

Abbreviations

5-OP-RU, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil; AHA, acute hepatitis A; ALT, alanine aminotransferase; CHB, chronic hepatitis B; FasL, Fas ligand; HDs, heathy donors; IFN, interferon; IL, interleukin; LSMCs, liver sinusoidal mononuclear cells; MAIT, mucosal-associated invariant T; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; MR1, MHC class I-related molecule 1; NK, natural killer; PB, peripheral blood; PBMCs, PB mononuclear cells; TCR, T cell receptor; TNF, tumor necrosis factor.

Financial support

This work was supported by Samsung Science and Technology Foundation under Project Number SSTF-BA1402-51.

Conflict of interest

The authors have no conflicting financial interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

M.-S.R., D.J.J., S.-H.P., and E.-C.S. designed the study. M.-S.R., J.W.H., J.H.K., and J.-Y.K. performed the experiments. H.J.P., S.I.K., M.S.K., J.G.L., D.H.L., W.K., J.Y.P., and D.J.J. provided clinical samples. M.-S.R., S.-H.P., and E.-C.S. analyzed the data and wrote the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.03.033.

References

Author names in bold designate shared co-first authorship

[1] Shin EC, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. Nat Rev Immunol 2016;16:509–523.
[2] Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. Nat Rev Immunol 2012;12:201–213.
[3] Jenne CN, Kubes P. Immune surveillance by the liver. Nat Immunol 2013;14:996–1006.
[4] Heymann F, Tacke F. Immunology in the liver–from homeostasis to disease. Nat Rev Gastroenterol Hepatol 2016;13:88–110.
[5] Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology 2006;43:S54–S62.
[6] Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. Hepatology 2008;47:729–736.
[7] Legoux F, Salou M, Lantz O. Unconventional or preset alphabeta T cells: evolutionarily conserved tissue-resident T cells recognizing nonpeptidic ligands. Annu Rev Cell Dev Biol 2017;33:511–535.
[8] Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. Nat Immunol 2015;16:1114–1123.
[9] **Treiner E**, **Duban L**, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature 2003;422:164–169.

[10] Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H, et al. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. J Exp Med 1999;189:1907–1921.
[11] **Dusseaux M**, **Martin E**, Serriari N, Peguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood 2011;117:1250–1259.
[12] **Tang XZ**, **Jo J**, **Tan AT**, **Sandalova E**, Chia A, Tan KC, et al. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. J Immunol 2013;190:3142–3152.
[13] **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;64:1118–1127.
[14] Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 2012;491:717–723.
[15] **Corbett AJ**, **Eckle SB**, **Birkinshaw RW**, **Liu L**, Patel O, Mahony J, et al. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. Nature 2014;509:361–365.
[16] Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 2010;11:701–708.
[17] **Le Bourhis L**, **Dusseaux M**, Bohineust A, Bessoles S, Martin E, Premel V, et al. MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS Pathog 2013;9:e1003681.
[18] Kurioka A, Ussher JE, Cosgrove C, Clough C, Fergusson JR, Smith K, et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. Mucosal Immunol 2015;8:429–440.
[19] **van Wilgenburg B**, **Scherwitzl I**, Hutchinson EC, Leng T, Kurioka A, Kulicke C, et al. MAIT cells are activated during human viral infections. Nat Commun 2016;7:11653.
[20] Slichter CK, McDavid A, Miller HW, Finak G, Seymour BJ, McNevin JP, et al. Distinct activation thresholds of human conventional and innate-like memory T cells. JCI Insight 2016;1:e86292.
[21] Loh L, Wang Z, Sant S, Koutsakos M, Jegaskanda S, Corbett AJ, et al. Human mucosal-associated invariant T cells contribute to antiviral influenza immunity via IL-18-dependent activation. Proc Natl Acad Sci U S A 2016;113:10133–10138.
[22] **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.
[23] **Jo J**, **Tan AT**, Ussher JE, Sandalova E, Tang XZ, Tan-Garcia A, et al. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. PLoS Pathog 2014;10:e1004210.
[24] Paquin-Proulx D, Avelino-Silva VI, Santos BAN, Silveira Barsotti N, Siroma F, Fernandes Ramos J, et al. MAIT cells are activated in acute Dengue virus infection and after in vitro Zika virus infection. PLoS Negl Trop Dis 2018;12:e0006154.
[25] **Kim J**, **Chang DY**, **Lee HW**, **Lee H**, Kim JH, Sung PS, et al. Innate-like cytotoxic function of bystander-activated CD8(+) T cells is associated with liver injury in acute hepatitis A. Immunity 2018;48:161–173.e5.
[26] Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. J Exp Med 2013;210:2305–2320.
[27] Abos B, Gomez Del Moral M, Gozalbo-Lopez B, Lopez-Relano J, Viana V, Martinez-Naves E. Human MR1 expression on the cell surface is acid sensitive, proteasome independent and increases after culturing at 26 degrees C. Biochem Biophys Res Commun 2011;411:632–636.
[28] Orange JS. Formation and function of the lytic NK-cell immunological synapse. Nat Rev Immunol 2008;8:713–725.
[29] Barber DF, Long EO. Coexpression of CD58 or CD48 with intercellular adhesion molecule 1 on target cells enhances adhesion of resting NK cells. J Immunol 2003;170:294–299.
[30] Mishra A, Sullivan L, Caligiuri MA. Molecular pathways: interleukin-15 signaling in health and in cancer. Clin Cancer Res 2014;20:2044–2050.
[31] Sattler A, Dang-Heine C, Reinke P, Babel N. IL-15 dependent induction of IL-18 secretion as a feedback mechanism controlling human MAIT-cell effector functions. Eur J Immunol 2015;45:2286–2298.
[32] **Meresse B**, **Chen Z**, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity 2004;21:357–366.
[33] Chu T, Tyznik AJ, Roepke S, Berkley AM, Woodward-Davis A, Pattacini L, et al. Bystander-activated memory CD8 T cells control early pathogen load in an innate-like, NKG2D-dependent manner. Cell Rep 2013;3:701–708.
[34] Correia MP, Stojanovic A, Bauer K, Juraeva D, Tykocinski LO, Lorenz HM, et al. Distinct human circulating NKp30(+)FcepsilonRIgamma(+)CD8(+) T cell population exhibiting high natural killer-like antitumor potential. Proc Natl Acad Sci U S A 2018;115:E5980–E5989.
[35] Crosby EJ, Goldschmidt MH, Wherry EJ, Scott P. Engagement of NKG2D on bystander memory CD8 T cells promotes increased immunopathology following Leishmania major infection. PLoS Pathog 2014;10:e1003970.
[36] Balin SJ, Pellegrini M, Klechevsky E, Won ST, Weiss DI, Choi AW, et al. Human antimicrobial cytotoxic T lymphocytes, defined by NK receptors and antimicrobial proteins, kill intracellular bacteria. Sci Immunol 2018;3:eaat7668.
[37] Kuylenstierna C, Bjorkstrom NK, Andersson SK, Sahlstrom P, Bosnjak L, Paquin-Proulx D, et al. NKG2D performs two functions in invariant NKT cells: direct TCR-independent activation of NK-like cytolysis and co-stimulation of activation by CD1d. Eur J Immunol 2011;41:1913–1923.
[38] Zumwalde NA, Haag JD, Gould MN, Gumperz JE. Mucosal associated invariant T cells from human breast ducts mediate a Th17-skewed response to bacterially exposed breast carcinoma cells. Breast Cancer Res 2018;20:111.
[39] Bierer BE, Peterson A, Gorga JC, Herrmann SH, Burakoff SJ. Synergistic T cell activation via the physiological ligands for CD2 and the T cell receptor. J Exp Med 1988;168:1145–1156.
[40] Springer TA, Dustin ML, Kishimoto TK, Marlin SD. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. Annu Rev Immunol 1987;5:223–252.
[41] Anikeeva N, Somersalo K, Sims TN, Thomas VK, Dustin ML, Sykulev Y. Distinct role of lymphocyte function-associated antigen-1 in mediating effective cytolytic activity by cytotoxic T lymphocytes. Proc Natl Acad Sci U S A 2005;102:6437–6442.
[42] Marcais A, **Cherfils-Vicini J**, **Viant C**, Degouve S, Viel S, Fenis A, et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol 2014;15:749–757.
[43] Nandagopal N, **Ali AK**, **Komal AK**, Lee SH. The critical role of IL-15-PI3K-mTOR pathway in natural killer cell effector functions. Front Immunol 2014;5:187.
[44] Leeansyah E, Svard J, Dias J, Buggert M, Nystrom J, Quigley MF, et al. Arming of MAIT cell cytolytic antimicrobial activity is induced by IL-7 and defective in HIV-1 infection. PLoS Pathog 2015;11:e1005072.
[45] Raulet DH, Gasser S, Gowen BG, Deng W, Jung H. Regulation of ligands for the NKG2D activating receptor. Annu Rev Immunol 2013;31:413–441.