Alcohol Abstinence Does Not Fully Reverse Abnormalities of Mucosal-Associated Invariant T Cells in the Blood of Patients With Alcoholic Hepatitis

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OBJECTIVES: Alcoholic hepatitis (AH) develops in approximately 30% of chronic heavy drinkers. The immune system of patients with AH is hyperactivated, yet ineffective against infectious diseases. Mucosal-associated invariant T (MAIT) cells are innate-like lymphocytes that are highly enriched in liver, mucosa, and peripheral blood and contribute to antimicrobial immunity. We aimed to determine whether MAIT cells were dysregulated in heavy drinkers with and without AH and the effects of alcohol abstinence on MAIT cell recovery.

METHODS: MR1 tetramers loaded with a potent MAIT cell ligand 5-(2-oxopropylideneamino)-6-d-ribofuranosyl-1-uracil were used in multiparameter flow cytometry to analyze peripheral blood MAIT cells in 59 healthy controls (HC), 56 patients with AH, and 45 heavy drinkers without overt liver disease (HDC) at baseline and 6- and 12-month follow-ups. Multiplex immunoassays were used to quantify plasma levels of cytokines related to MAIT cell activation. Kinetic Turbidimetric Limulus Amebocyte Lysate Assay and ELISA were performed to measure circulating levels of 2 surrogate markers for bacterial translocation (lipopolysaccharide and CD14), respectively.

RESULTS: At baseline, patients with AH had a significantly lower frequency of MAIT cells than HDC and HC. HDC also had less MAIT cells than HC (median 0.16% in AH, 0.56% in HDC, and 1.25% in HC). Further, the residual MAIT cells in patients with AH expressed higher levels of activation markers (CD69, CD38, and human leukocyte antigen [HLA]-DR), the effector molecule granzyme B, and the immune exhaustion molecule PD-1. Plasma levels of lipopolysaccharide and CD14 and several cytokines related to MAIT cell activation were elevated in patients with AH (interferon [IFN]-γ, interleukin [IL]-7, IL-15, IL-17, IL-23, IFN-γ, and tumor necrosis factor α). Decreased MAIT cell frequency and upregulated CD38, CD69, and HLA-DR correlated negatively and positively, respectively, with aspartate aminotransferase level. MAIT cell frequency negatively correlated with IL-18. HLA-DR and CD38 levels correlated with several cytokines. At follow-ups, abstinent patients with AH had increased MAIT cell frequency and decreased MAIT cell activation. However, MAIT cell frequency was not fully normalized in patients with AH (median 0.31%).

DISCUSSION: We showed that HDC had a reduction of blood MAIT cells despite showing little evidence of immune activation, whereas patients with AH had a severe depletion of blood MAIT cells and the residual cells were highly activated. Alcohol abstinence partially reversed those abnormalities.

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A52, http://links.lww.com/CTG/A53, and http://links.lww.com/CTG/A54

Clinical and Translational Gastroenterology 2019;10:e-00052. https://doi.org/10.14309/ctg.0000000000000052

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INTRODUCTION

Excessive alcohol consumption causes hepatocellular injury through direct cytotoxic effects and oxidative stress mediated by ethanol and its metabolites and induction of proinflammatory cascades. Up to a third of long-term heavy drinkers develop a spectrum of severe alcoholic liver disease (ALD), ranging from alcoholic hepatitis (AH), fibrosis/cirrhosis, to hepatocellular carcinoma. Approximately 10%–35% of the heavy drinking population develops AH, a severe and progressive acute-on-chronic liver inflammation disease with significant morbidity and mortality, for which there are limited treatment options (1–3). AH is clinically characterized by hyperbilirubinemia, coagulopathy, elevation of liver enzyme levels, and features of the systemic inflammatory response syndrome in heavy drinkers with a history of recent alcohol abuse. Although the exact trigger for development of AH is still not well-understood, alcohol-induced dysregulation of both innate and adaptive immune systems has been implicated in the pathogenesis of AH (4–7).

Excessive drinking leads to dysbiosis of gut microbiome (8–10), which contributes to alcohol-induced breakdown of gastrointestinal (GI) tract barrier. Increased intestinal permeability results in the translocation of microbes and microbial products from the GI tract into blood, which reach the liver through the portal vein, contributing to alcohol-induced breakdown of gastrointestinal (GI) tract barrier. Increased intestinal permeability results in which contributes to alcohol-induced breakdown of gastrointestinal (GI) tract barrier. Increased intestinal permeability results in the translocation of microbes and microbial products from the GI tract into blood, which reach the liver through the portal vein, which contributes to alcohol-induced breakdown of gastrointestinal (GI) tract barrier. Increased intestinal permeability results in the translocation of microbes and microbial products from the GI tract intoblood, which reach the liver through the portal vein, which contributes to alcohol-induced breakdown of gastrointestinal (GI) tract barrier. Increased intestinal permeability results in

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## Table 1. Comparison of characteristics of patients with AH and HDC in the TREAT study cohort

| Variable                        | HC (n = 59) | AH (n = 56) | HDC (n = 45) | P     | AH (n = 24) | HDC (n = 25) | P     | AH (n = 15) | HDC (n = 22) | P     |
|--------------------------------|-------------|-------------|--------------|-------|-------------|--------------|-------|-------------|--------------|-------|
| Age at enrollment (yr)         | 41 ± 14     | 45 ± 10     | 43 ± 10      | ns    | 43 ± 10     | 43 ± 10      | ns    | 46 ± 11     | 46 ± 10      | ns    |
| Gender (% male)                | 53          | 61          | 64           | ns    | 63          | 64           | ns    | 53          | 68           | ns    |
| Race (% white)                 | 71          | 95          | 78           | ns    | 95          | 80           | ns    | 100         | 64           | **    |
| Total drinks in the last 30 d  | 237 ± 221   | 412 ± 409   | *            |       | 4 ± 12      | 26 ± 61      | ns    | 12 ± 46     | 80 ± 188     | ns    |
| Total drinking d in the last 30 d | 22 ± 10   | 25 ± 7      | ns           |       | 1 ± 4       | 4 ± 8        | ns    | 2 ± 8       | 10 ± 14       | ns    |
| MELD score                     | 25 ± 7      | 7 ± 2       | ***          |       | 15 ± 9      | 7 ± 1        | ***   | 12 ± 6      | 7 ± 1         | ***   |
| Creatinine (mg/dL)             | 0.9 ± 0.2\* | 1 ± 0.7     | 0.9 ± 0.3    | ns    | 1 ± 0.8     | 1 ± 0.2      | ns    | 1 ± 0.4     | 1 ± 0.2       | ns    |
| Total bilirubin (mg/dL)        | 0.5 ± 0.2\* | 18.1 ± 12.8### | 0.6 ± 0.3    | ***   | 3.6 ± 4.1   | 0.4 ± 0.2    | ***   | 2.4 ± 3.9   | 0.5 ± 0.2     | ***   |
| AST (IU/L)                     | 18 ± 6\*    | 123 ± 55### | 26 ± 9\$§    | ***   | 59 ± 49     | 20 ± 6       | ***   | 42 ± 29     | 30 ± 30       | **    |
| ALT (IU/L)                     | 14 ± 7\*    | 50 ± 27###  | 25 ± 10\$§§  | ***   | 35 ± 22     | 17 ± 8       | ***   | 26 ± 14     | 26 ± 19       | ns    |
| Prothrombin time (INR)         | 1.9 ± 0.5   | 1 ± 0.1     | ***          |       | 1.5 ± 0.6   | 1 ± 0.1      | ***   | 1.3 ± 0.5   | 1 ± 0.1       | ***   |

Data are represented as mean ± SD. Kruskal-Wallis test with Dunn’s correction for pairwise comparisons of continuous variables among HC, patients with AH, and HDC at enrollment (day 0). Mann-Whitney test comparing patients with AH vs HDC at 180-day and 360-day follow-up. Chi-square test for analysis of categorical variables.

AH, alcoholic hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HC, healthy controls; HDC, heavy drinking controls; INR, international normalized ratio; MELD, model for end-stage liver disease; ns, not significant.

\*Data from 44 HC.

### P < 0.01 for comparison between patients with AH and HC at day 0; \* P < 0.05; \** P < 0.01; \*** P < 0.001 for comparison between patients with AH and HDC; §§ P < 0.01; §§§ P < 0.001 for comparison between HDC and HC at day 0.
patients with AH and 25 HDC at 180-day follow-up, and 15 patients with AH and 22 HDC at 360-day follow-up) were part of the multicenter prospective Translational Research and Evolving Alcoholic Hepatitis Treatment 001 study (TREAT 001, NCT02172898). Demographic and clinical characterizations as well as drinking patterns of the study subjects are shown in Table 1. Detailed definitions of AH and HDC and the inclusion and exclusion criteria were previously described (39). HDC were individuals with a comparable history of alcohol consumption but had no clinical evidence of liver disease (aspartate aminotransferase [AST] ≤ 50 U/L, alanine aminotransferase [ALT] ≤ 50 U/L, and total bilirubin within normal limits) and were matched for age, sex, and race. Individuals with liver diseases of other etiologies and clinically overt and active infection were excluded from this study.

Blood samples
Peripheral blood was collected in heparin-coated tubes (BD Biosciences, Franklin Lakes, NJ) and separated into plasma and peripheral blood mononuclear cells (PBMCs). Both plasma and PBMCs were stored at −80 °C until use. PBMC and plasma samples from 59 age-, sex-, and race-matched healthy volunteers were included as HC.

Flow cytometry
PBMCs were subjected to cell surface staining and intracellular staining to determine leukocyte frequency and phenotype. For cell surface staining, PBMCs were incubated with fixable viability dye (ThermoFisher Scientific, Waltham, MA) to discriminate live and dead cells, followed by staining with fluorochrome-conjugated antibodies against human CD3, CD4, CD8, CD161, CD38, CD56, CD69, HLA-DR, gamma delta T (γδ T) cells, invariant natural killer T (iNKT) cells (all from BioLegend, San Diego, CA), and human MR1 tetramers loaded with a potent MAIT cell ligand 5-OP-RU (5-(2-oxopropylideneamino)-6-d-ribitylaminouracil). Appropriate isotype controls were used at the same protein concentration as the test antibodies for control staining, and human MR1 tetramers loaded with 6-FP (6-formylpterin) were used as a negative control for MAIT cell staining. Human MR1 tetramers loaded with 5-OP-RU or 6-FP were produced by the NIH Tetramer Core Facility as permitted for distribution by the University of Melbourne. Cells stained with surface markers were fixed and permeabilized with the Cytofix/Cytoperm reagents (BD Biosciences, San Jose, CA). For intracellular staining, fixed cells were permeabilized with the Cytofix/Cytoperm reagents (BD Biosciences), followed by staining with antibodies against granzyme B (BioLegend) and active form of caspase 3 (BD Biosciences). Cells were subsequently acquired using a BD LSRSFortessa flow cytometer (BD Biosciences). Flow data were analyzed using FlowJo v10 software (Tree Star, San Carlos, CA).

Plasma endotoxin and soluble CD14
Plasma levels of the endotoxin LPS from Gram-negative bacteria and soluble CD14 (sCD14) were used as surrogate markers for bacterial translocation from gut to circulation. LPS amounts were determined using the PYROGENT-5000 Kinetic Turbidimetric Limulus Amebocyte Lysate Assay (Lonza, Walkersville, MD). Plasma samples were diluted 5-folds in endotoxin-free water and heated at 70 °C for 20 minutes before being subjected to the assay. Plasma sCD14 levels were quantified using the Human CD14 Quantikine kit (R&D Systems, Minneapolis, MN).

Multiplex immunoassays
The Cytokine/Chemokine/Growth Factor 45-Plex Human ProcartaPlex Panel 1 (ThermoFisher Scientific) was used to simultaneously measure plasma concentrations of 45 proteins (BDNF, Eotaxin/CCL11, EGF, FGF-2, GM-CSF, GROα/CXCL1, HGF, NGF-β, LIF, IFN-α, IFN-γ, IL-1β, IL-1α, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12 p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, regulated upon activation normal T cell expressed and secreted [RANTES]/CCL5, stromal cell-derived factor [SDF]-1α/CXCL12, TNF-α, TNF-β/LTA, platelet-derived growth factor [PDGF]-BB, placenta Growth Factor [PLGF], stem-cell factor [SCF], vascular endothelial growth factor [VEGF]-A, and VEGF-D). Multiplex assay was conducted as previously described (17). The concentrations of cytokines/chemokines were calculated using the Bio-Plex Manager v6.1 software (Bio-Rad, Hercules, CA). For statistical analyses, values below the detection limit of the assay were replaced with the minimal detectable concentrations for each analyte as provided by the manufacturer.

Statistical analysis
Differences in cross-sectional analysis for continuous variables between 2 groups were calculated using Mann-Whitney test and Kruskal-Wallis test with Dunn’s corrections for comparisons among 3 groups. Chi-square test was used for comparison between groups for categorical variables. The linear relationship between 2 variables was analyzed using the Spearman correlation test. Differences in longitudinal analysis were calculated using Friedman rank sum test with Dunn’s corrections. P < 0.05 was considered statistically significant.

RESULTS
Characteristics of the study cohort
Table 1 summarizes the demographic and clinical characteristics of 56 patients with AH, 45 HDC, and 59 HC at enrollment; 24 patients with AH and 25 HDC at 6-month follow-up; and 15 patients with AH and 22 HDC at 12-month follow-up. There were no differences in age, gender, and race distributions and creatinine levels among the 3 groups at baseline and between patients with AH and HDC at follow-up. Although the amounts of baseline total bilirubin were similar between HDC and HC, both AST and ALT levels were modestly and significantly higher in HDC than HC. The baseline model for end-stage liver disease (MELD) score or liver biochemistries (AST, ALT, and total bilirubin) were highly elevated in patients with AH as compared to HDC and HC. Prothrombin time was also significantly longer for the patients with AH than HDC. Interestingly, the HDC had significantly more drinks than patients with AH before the enrollment. At the follow-up, both patients with AH and HDC drank much less and the liver biochemistries greatly improved in AH patients and remained unchanged for HDC. However, patients with AH still had a significantly higher MELD score due to elevated levels of liver biochemistries and prolonged prothrombin time. The complete abstinence rate was 79% and 87% for patients with AH and 68% and 59% for HDC at 6 and 12 months, respectively. The MELD score, total bilirubin, AST, and prothrombin time remained higher in abstinent patients with AH at 6 and 12 months compared to abstinent HDC (Supplementary Table 1, Supplementary Digital Content 3, http://links.lww.com/CTG/A54). However, ALT levels were similar between patients with AH and HDC at 12-month follow-up (Supplementary Table 1, Supplementary
Circulating MAIT cells were severely depleted in patients with AH. To analyze the impact of chronic heavy drinking on peripheral MAIT cells, we first analyzed the frequency of MAIT cells in peripheral blood in patients with AH and HDC as compared to HC. MAIT cells were identified as CD3⁺ lymphocytes that express high level of CD161 and are stained by the 5-OP-RU-loaded MR1 tetramers (40). The staining specificity was verified by the negative control MR1-6-FP tetramers. At baseline, MAIT cells were significantly depleted in patients with AH as compared to HC and HDC (Figure 1a,b). HDC also had significantly less MAIT cells than HC (Figure 1a,b). Specifically, MAIT cells accounted for a median of 0.16% (range: 0.03%–1.9%; interquartile range [IQR]: 0.09%–0.34%), 0.56% (range: 0.03%–7.22%; IQR: 0.23%–1.41%), and 1.25% (range: 0.05%–5.17%; IQR: 0.63%–2.32%) T cells in patients with AH, HDC, and HC, respectively. This decrease of peripheral MAIT cells was not due to enhanced cell death, because there was no increase in active form of caspase 3, a marker for apoptosis (Figure 1c) or down-regulation of CD161 for MAIT cells in patients with AH or HDC (Figure 1a). As a comparison, the frequency of CD161-expressing non-MAIT T cells, which contain highly proinflammatory CD4⁺ and CD8⁺ cells (41), was not decreased, but significantly increased in patients with AH compared to HC and HDC (Figure S1A, Supplementary Digital Content 1, http://links.lww.com/CTG/A52).
In homeostasis, the majority of MAIT cells are CD8-positive, with a small percentage being CD4-positive, or DN for CD4 and CD8 (42). There was a significant decrease of CD8-expressing MAIT cells, and a corresponding increase in CD4-expressing MAIT cells in patients with AH as compared to HDC and HC (Figure S1B/S1C, Supplementary Digital Content 1, http://links.lww.com/CTG/A52). The frequency of DN cells was similar among patients with AH, HDC, and HC (Figure S1D, Supplementary Digital Content 1, http://links.lww.com/CTG/A52). There were no significant differences in the frequency of several other types of innate T cells, including NKT, iNKT, and γδ T cells, among patients with AH, HDC, and HC (Figure S2, Supplementary Digital Content 2, http://links.lww.com/CTG/A53).

Patients with AH tended to have lower frequency of MAIT cells than HDC at 6-month follow-up ($P = 0.08$) and at 12-month follow-up ($P = 0.10$) (Figure 1b). Furthermore, frequency of MAIT cells at 12-month follow-up in both patients with AH (median: of 0.31%; range: 0.04%–1.45%; IQR: 0.17%–0.46%) and...
Table 2. Comparison of levels of inflammatory factors related to MAIT cell activation

| Variable     | Stimulation related factors | Day 0 (baseline) | Day 180 | Day 360 |
|--------------|----------------------------|-----------------|---------|---------|
|              | HC (n = 43–47)              | AH (n = 51–55)  | HDC (n = 40–43) | AH (n = 18–21) | HDC (n = 19–23) | AH (n = 13–14) | HDC (n = 15–17) |
|              |                             |                 |         |         |
| LPS (EU/mL)  | 0.01 ± 0.01###              | 2.78 ± 10.34*** | 0.02 ± 0.04 | 38.83 ± 119.3 | 0.02 ± 0.02 | 0.11 ± 0.33 | 0.04 ± 0.06 |
| sCD14 (ng/mL)| 1,391 ± 530###              | 2,489 ± 1,110***| 1,619 ± 462 | 1,435 ± 394 | 1,269 ± 256 | 1,697 ± 505 | 1,405 ± 328 |
| IFN-α (pg/mL)| 1.1 ± 1.6                  | 3.4 ± 4.7**     | 1.3 ± 3 | 3.4 ± 5.4 | 3 ± 6.2 | 3.6 ± 5.7 | 2.7 ± 5.8 |
| IL-7 (pg/mL) | 0.5 ± 1##                   | 2.1 ± 3.3**     | 0.5 ± 1.3 | 1.5 ± 1.8** | 0.4 ± 0.4 | 0.6 ± 0.4 | 0.6 ± 0.8 |
| IL-12 (pg/mL)| 1.3 ± 0.5###                | 2.1 ± 1.8      | 1.5 ± 0.4 | 1.8 ± 0.7** | 1.2 ± 0.6 | 1.4 ± 0.6 | 1.1 ± 0.6 |
| IL-15 (pg/mL)| 18.2 ± 37                  | 41.8 ± 73.2*    | 22.4 ± 59.4 | 65.2 ± 116.5* | 59 ± 144.2 | 73 ± 144.2 | 55.9 ± 149.3 |
| IL-18 (pg/mL)| 15.4 ± 15.9###             | 87.7 ± 91***   | 26.5 ± 37.7 | 66.8 ± 75.8** | 21 ± 17.9 | 54.7 ± 49.3** | 24.3 ± 12.9 |
| IL-23 (pg/mL)| 35.6 ± 35###                | 115.7 ± 204*** | 25.3 ± 14.9 | 65.8 ± 42.7*** | 21.2 ± 23.8 | 56 ± 47.4* | 28.5 ± 25.7 |
| Effectors    |                             |                 |         |         |
| IFN-γ (pg/mL)| 4.9 ± 5.1###               | 32.1 ± 33.8*** | 9.2 ± 10.6 | 23.2 ± 21.3* | 9.1 ± 12.2 | 19.2 ± 14.1* | 10.3 ± 12.5 |
| IL-17 (pg/mL)| 0.8 ± 1.8##                | 15.3 ± 32**    | 0.7 ± 6.1 | 2.3 ± 2.3* | 1.5 ± 3.3 | 1.5 ± 2 | 1.5 ± 3.8 |
| TNF-α (pg/mL)| 6.5 ± 6.1###               | 14.8 ± 18.1*** | 8.3 ± 10.2 | 20.3 ± 16.9* | 11.6 ± 11.5 | 14.6 ± 14.5 | 9.9 ± 11.9 |

Data are represented as mean ± SD. Kruskal-Wallis test with Dunn’s correction for pairwise comparisons among HC, patients with AH, and HDC at baseline (day 0). Mann-Whitney test comparing patients with AH vs HDC at 180-day and 360-day follow-up. AH, alcoholic hepatitis; HC, healthy controls; HDC, heavy drinking controls; IFN, interferon; IL, interleukin; LPS, lipopolysaccharides; MAIT, mucosal-associated invariant T cell; sCD14, soluble CD14; TNF, tumor necrosis factor. ##p < 0.01; ###p < 0.001 for comparison between patients with AH and HC at day 0; *P < 0.05; **P < 0.01; ***P < 0.001 for comparison between AH patients and HDC.
HDC (median: 0.61%; range: 0.03%–4.05%; IQR: 0.17%–1.37%) was still significantly lower than that in HC (Figure 1b). However, the percentage of CD8^+^, CD4^+^, DN, and caspase 3^+^ MAIT cells and CD161^+^ non-MAIT cells was not different between the AH and HDC groups at the follow-up. Thus, at baseline, MAIT cells were selectively depleted in patients with AH, and the remaining MAIT cells had altered CD4 and CD8 T cell coreceptor expression. There was a partial, but not complete, recovery of MAIT cells at follow-up in patients with AH.

Residual MAIT cells in patients with AH expressed higher levels of activation markers

We have recently reported that the CD4 and CD8 T cells from patients with AH are highly dysregulated, expressing higher activation markers such as CD69 and CD38 than T cells from HDC (17). Here, we examined whether MAIT cells in patients with AH were also hyperactivated. Compared with HDC and HC, significantly higher percentages of MAIT cells from patients with AH expressed CD69 and CD38, whereas those markers were not significantly elevated in MAIT cells from HDC relative to HC (Figure 2a,b). MAIT cells from patients with AH also expressed significantly more HLA-DR (another T-cell activation marker) than HC, whereas the frequencies of CD38 and HLA-DR double-positive cells (often used as an indicator of T-cell activation in chronic inflammation) were not different among the heavy drinkers and HC (Figure 2c,d). Consistent with expressing higher levels of surface activation markers, MAIT cells from patients with AH also contained significantly more granzyme B than HC, but not more than HDC (Figure 2e). T cells in patients with AH express higher levels of several immune checkpoint inhibitory or exhaustion molecules such as TIM3 and PD-1 (16). As shown in Figure 2f, a higher percentage of MAIT cells from patients with AH expressed PD-1 as compared to HC, and the difference between the AH and HDC groups was not significant. Further cross-sectional analyses of follow-up samples showed that only CD38 expression on MAIT cells was still higher in patients with AH than HDC at 6-month follow-up, and the expression of all those markers on MAIT cells was not significantly different between the AH and HDC groups (Figure 2c–f). Taken together, our results indicated that blood MAIT cells in patients with AH were highly activated and exhausted. These cellular abnormalities were reversed at 12-month follow-up, which corresponded with reduced alcohol consumption.

Soluble factors related to MAIT cell activation were elevated in patients with AH

MAIT cells recognize and are activated by MR1 receptors complexed with vitamin B2 biosynthesis precursor derivatives from

| Table 3. Correlations of MAIT cell markers with clinical and inflammatory factors in patients with AH |
|-------------------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Variable                  | % MAIT | % CD69^+^ | % CD38^+^ | % HLA-DR^+^ | % GRB^+^ | % PD-1^+^ |
| Clinical parameters       |        |         |            |              |            |            |
| MELD                      |        |         |            |              |            |            |
| Creatinine                |        |         |            |              |            |            |
| Total bilirubin           |        |         |            |              |            |            |
| AST                       | −0.36**| 0.35**  | 0.28*      | 0.3*         |          |          |
| ALT                       |        |         |            |              |            |            |
| INR                       |        |         |            |              |            |            |
| Stimulation-related factors|        |         |            |              |            |            |
| LPS                       |        |         |            |              |            |            |
| sCD14                     | 0.38** |         |            |              | 0.48***    |            |
| IFN-α                     |        | 0.46*** |            |              |            |            |
| IL-7                      |        | 0.35*   | 0.4**      |              | 0.33*      |            |
| IL-12                     |        | 0.4**   |            |              |            |            |
| IL-15                     |        | 0.51*** | 0.3*       |              |            |            |
| IL-18                     | −0.36**| 0.37**  | 0.29*      |              |            |            |
| IL-23                     |        |         |            |              | 0.31*      |            |
| Effectors                 |        |         |            |              |            |            |
| IFN-γ                     |        | 0.41**  | 0.33*      |              |            |            |
| IL-17                     |        | 0.36*   |            |              |            |            |
| TNF-α                     |        | 0.54*** | 0.56***    |              |            |            |

The numbers represent Spearman’s coefficients. Negative numbers represent negative correlations. AH, alcoholic hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GRB, granzyme B; IFN, interferon; IL, interleukin; INR, international normalized ratio for prothrombin time; LPS, lipopolysaccharides; MELD, model for end-stage liver disease; MAIT, mucosal-associated invariant T cell; sCD14, soluble CD14; TNF, tumor necrosis factor.

*^P < 0.05; **^P < 0.01; ***^P < 0.001.
a wide range of Gram-negative and Gram-positive bacteria. In AH, impaired integrity of the GI tract leads to translocation of bacterial components to circulation. We therefore measured circulatory levels of LPS derived from Gram-negative bacteria and sCD14, a receptor for LPS that is secreted by activated macrophages and monocytes, as a marker for bacterial translocation. As shown in Table 2, the baseline LPS and sCD14 levels were elevated in patients with AH in comparison to HDC and HC, whereas levels of those factors were not significantly upregulated in HDC compared to HC. At 6- and 12-month follow-ups, the amounts of both factors were similar between the AH and HDC groups.

MAIT cells can also be activated in a TCR-independent manner by cytokines, such as IL-18, IL-12, IL-15, IFN-α, IL-23, IL-7, to produce effector cytokines (IFN-γ, IL-17, and TNF-α), which might further enhance expression of MAIT cell activation markers. As shown in Table 2, the baseline LPS and sCD14 levels were elevated in patients with AH in comparison to HDC and HC, whereas levels of those factors were not significantly upregulated in HDC compared to HC. At 6- and 12-month follow-ups, the amounts of both factors were similar between the AH and HDC groups.

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Association of MAIT cell frequency and phenotype with clinical profiles and cytokine levels in patients with AH
To explore whether MAIT cells might be related to pathogenesis of AH, we determined whether MAIT cell frequency and the activation markers correlated with disease severity represented by clinical scores (MELD score) and biochemical measurements (creatinine, total bilirubin, AST, ALT) and prothrombin time in patients with AH at baseline. Although MAIT cell frequency and phenotype did not correlate with the MELD score, the frequencies of MAIT cells and MAIT cell activation markers CD69, CD38, and HLA-DR correlated negatively and positively with level of the liver enzyme AST, respectively, suggesting dysregulated MAIT cells might be
involved in liver damage. The percentage of PD-1-positive MAIT cells correlated with the creatinine level (Table 3).

Next, we analyzed the correlations between MAIT cell phenotype and factors that are linked to MAIT cell activation. The significant correlations were listed in Table 3. Circulatory levels of LPS did not correlate with MAIT frequency or activation phenotype, whereas sCD14 level correlated with percentages of CD69$^+$ and granzyme B$^+$ MAIT cells. IL-18 was negatively associated with MAIT frequency and positively with CD38$^+$ (%) and HLA-DR$^+$ (%) MAIT cells. Other MAIT-stimulatory cytokines (IL-7, IL-12, IL-15, and IL-23) also correlated with CD38$^+$ (%) and HLA-DR$^+$ (%) MAIT cells. Effector cytokines IL-17, IFN-γ, and TNF-α also showed significant correlations with the frequency of MAIT activation markers CD38 and HLA-DR. Interestingly, the frequency of PD-1$^+$ MAIT cells in the patients with AH inversely correlated with the percentage of MAIT cells ($r = 0.41, P = 0.003$) as well as CD8$^+$ MAIT cells ($r = 0.35, P = 0.01$). These results suggested that stimulation by bacterial products and multiple cytokines could contribute to MAIT cell hyperactivation and that immune exhaustion might play a role in MAIT cell depletion.

**Loss of MAIT cells was not fully reversed in patients with AH by alcohol abstinence**

To determine whether the partial recovery of MAIT abnormalities at follow-up was linked to alcohol abstinence in patients with AH, we performed cross-sectional analysis on follow-up samples between abstinent patients with AH and HDC. As shown in Figure 3a,b, the frequencies of MAIT cells and CD38$^+$ MAIT cells were still significantly lower and higher, respectively, in the AH group at 6-month follow-up. Other MAIT cell activation markers, including CD69, HLA-DR, and HLA-DR$^+$, showed significant correlations with the frequency of MAIT cells and CD38$^+$ MAIT cells (Table 3).
Peripheral MAIT Cells in Patients with Alcoholic Hepatitis

In the present study, we performed cross-sectional and longitudinal analysis to compare frequency, phenotype, and function of peripheral blood MAIT cells from a large cohort of patients with AH, matched heavy drinkers, and HC (Table 1). The design of our study allowed us to identify changes in MAIT cells related to heavy drinking alone, during AH developments, and immune system recovery following alcohol abstinence. We demonstrated that patients with AH had a severe reduction of MAIT cells and the residual MAIT cells exhibited a highly activated and exhausted phenotype (Figures 1 and 2). On the other hand, HDC only had a slight decrease in MAIT cell frequency and the remaining MAIT cells did not exhibit significant upregulation of activation markers compared to HC. Baseline plasma levels of markers of bacterial translocation to circulation (LPS and sCD14) and several cytokines linked to MAIT cell activation (IFN-α, IL-7, IL-12, IL-15, IL-18, IL-23, IFN-γ, IL-17, and TNF-α) were highly elevated in patients with AH (Table 2). Baseline MAIT cell frequency and levels of MAIT activation markers CD69, CD38, and HLA-DR correlated negatively and positively with AST in patients with AH, respectively. MAIT cell frequency was also negatively associated with IL-18, whereas up-regulated CD38 and HLA-DR showed positive correlations with many of the cytokines implicated in MAIT cell activation (Table 3). Analysis of follow-up and longitudinal samples indicated MAIT cell abnormalities in patients with AH drastically improved but were not completely reversed with alcohol cessation (Figures 3 and 4), which was reflected by changes in their clinical characteristics.

Next, we performed longitudinal analysis of samples from patients with AH and HDC who were completely abstinent at follow-up. As shown in Figure 4a, MAIT cell frequency in patients with AH was significantly increased at 12-month but not 6-month follow-up; MAIT cells from abstinent patients with AH had significant reductions in CD69 and CD38 expression at 6-month follow-up and in CD38 expression at 12-month (Figure 4a). In contrast, frequencies of MAIT cells and those activation markers in abstinent HDC did not have significant changes throughout the study (Figure 4b). Taken together, our data indicated that the dysregulated MAIT cell frequency and MAIT activation markers in patients with AH recovered greatly, but not completely, with alcohol cessation.

DISCUSSION

In the present study, we performed cross-sectional and longitudinal analysis to compare frequency, phenotype, and function of peripheral blood MAIT cells from a large cohort of patients with AH, matched heavy drinkers, and HC (Table 1). The design of our study allowed us to identify changes in MAIT cells related to heavy drinking alone, during AH developments, and immune system recovery following alcohol abstinence. We demonstrated that patients with AH had a severe reduction of MAIT cells and the residual MAIT cells exhibited a highly activated and exhausted phenotype (Figures 1 and 2). On the other hand, HDC only had a slight decrease in MAIT cell frequency and the remaining MAIT cells did not exhibit significant upregulation of activation markers compared to HC. Baseline plasma levels of markers of bacterial translocation to circulation (LPS and sCD14) and several cytokines linked to MAIT cell activation (IFN-α, IL-7, IL-12, IL-15, IL-18, IL-23, IFN-γ, IL-17, and TNF-α) were highly elevated in patients with AH (Table 2). Baseline MAIT cell frequency and levels of MAIT activation markers CD69, CD38, and HLA-DR correlated negatively and positively with AST in patients with AH, respectively. MAIT cell frequency was also negatively associated with IL-18, whereas up-regulated CD38 and HLA-DR showed positive correlations with many of the cytokines implicated in MAIT cell activation (Table 3). Analysis of follow-up and longitudinal samples indicated MAIT cell abnormalities in patients with AH drastically improved but were not completely reversed with alcohol cessation (Figures 3 and 4), which was reflected by changes in their clinical characteristics.

Figure 5. Model of MAIT cell activation and depletion in patients with AH. Chronic heavy drinking induces dysbiosis of gut microbiome and breakdown of GI tract barrier, which leads to translocation of gut bacteria and their products to the portal and systemic circulation in patients with AH. Bacterial products, such as LPS, activate APC and other cells to release MAIT cell-activating cytokines, including IL-18, IL-7, IL-15, and IL-23, and IFN-α. In addition, bacterial-derived vitamin B2 metabolites presented by the major histocompatibility complex class I-related protein 1 (MR1) on APC activate MAIT cells through the invariant Vα7.2-Jα33 T-cell receptor. Both the cytokine- and antigen-dependent signaling pathways of MAIT cell activation contribute to upregulation of T-cell activation markers (CD69, CD38, and HLA-DR), the T-cell exhaustion marker PD-1, and production of cytotoxic effector proteins (granzyme B and perforin), and effector cytokines (IFN-γ, IL-17, and TNF-α, which can serve as a positive feedback to further activate MAIT cells). These hyperactivated and exhausted MAIT cells are prone to activation-induced cell death, leading to depletion of hepatic and circulating MAIT cells and impaired antimicrobial cytokine and cytotoxic responses for the residual MAIT cells in patients with AH. AH, alcoholic hepatitis; APC, antigen-presenting cells; GI, gastrointestinal; LPS, lipopolysaccharides; MAIT, mucosal-associated invariant T cell; TNF, tumor necrosis factor.
Peripheral blood MAIT cells were markedly depleted in patients with AH at enrollment as quantified by MR1-5-OP-RU tetramer staining. This result confirmed a previous finding that blood MAIT cells are profoundly reduced in patients with severe AH using CD161hi-TCR-Vα7.2+ phenotyping to identify MAIT cells (37). On the other hand, we did not find major changes in several other innate T-cell populations, such as NKT, iNKT, and γδ T cells, indicating that MAIT cells were specifically depleted in AH. CD8+ MAIT cells have been identified as the dominant subset of MAIT cells. We and others have shown that patients with AH have reduced frequency of total CD8 T cells in circulation (5,17,19). In line with this general reduction of CD8 T cells in peripheral blood, we found that the frequency of CD8+ MAIT cells was reduced with a corresponding increase in CD4+ MAIT cells. Similar decrease in CD8+ MAIT cells and increase in CD4+ MAIT cells has been observed in several chronic liver diseases, including ALD (35). In addition to overt cell exhaustion, the loss of peripheral MAIT cells could be partly due to redistribution to the liver, although the existing data are contradictory. One study has reported that CD161hi-TCR-Vα7.2+ MAIT cells dramatically depleted in the blood, but the TCR-Vα7.2+ cells (also include non-MAIT cells) were preserved in the liver of patients with ARC (37). However, 2 other studies have demonstrated that frequency of CD161hi-TCR-Vα7.2+ MAIT cells are drastically reduced in both the blood and liver of patients with end-stage ALD (35,43). Thus, further study with MR1 tetramers to quantify MAIT cells in liver biopsies from patients with AH is required to assess whether blood MAIT cell depletion results from MAIT cell recruitment into the liver or dysregulation of cell homeostasis and survival.

MAIT cell depletion from the periphery, which is a common occurrence in many infectious and inflammatory diseases, is concurrent with upregulation of activation and exhaustion markers (19,32,34). Hyperactivation often leads to activation-induced cell death by apoptosis. Human MAIT cells are known to have a proapoptotic phenotype in response to cell activation (44). MAIT cells in this large cohort of patients with AH expressed high levels of activation markers CD69, CD38, HLA-DR, and the immune inhibitory/exhaustion marker PD-1. This is in line with findings showing that hyper-activated MAIT cells in other diseases also express higher levels of inhibitory molecules PD-1 and TIM3 (19). However, a recent study found no increase in expression levels of 3 immune checkpoint inhibitors (PD-1, TIM3, and LAG3) in 9 patients with AH and ARC (37). In our study, we did not observe an increase in apoptotic MAIT cells or non-MAIT T cells in patients with AH or HC. It is possible that apoptotic MAIT cells were rapidly cleared from the circulation before sample collection or the cell death was independent of apoptosis.

In TCR-dependent activation, MAIT cells are activated by MR-1 complexes with bacterial-derived vitamin B2 metabolites. In AH, bacterial components and metabolites are released from gut to circulation due to increased intestinal permeability (11,45). We used LPS and sCD14 as surrogate markers for gut bacterial translocation. Both markers were significantly higher in patients with AH than HDC and HC, which is consistent with the results recently reported (9). Interestingly, fecal extracts containing gut bacteria and bacterial antigens and metabolites (FEB) from patients with severe AH and ARC, but not FEB from healthy donors or fixed laboratory strain of Escherichia coli, can induce depletion and dysfunction of MAIT cells from PBMCs of healthy donors (37). Although the underlying mechanism for this observation is unknown, dysbiosis of gut microbiome likely plays a role. Recently, dysbiosis of the circulating microbiome in patients with AH as well as in HDC has been shown as compared to nondrinking controls (9). The relationship between dysbiosis of gut and blood microbiome and MAIT cell dysregulation requires further investigation.

Of the several MAIT cell activation-related cytokines that were upregulated in patients with AH, we found that multiple inflammatory cytokines correlated with expression of the T-cell activation markers CD38 and HLA-DR, which likely reflected hyperinflammation in AH. Interestingly, we found that IL-18 level negatively correlated to MAIT frequency, suggesting that IL-18 likely plays a role in MAIT cell depletion in patients with AH. Currently, the relative contribution of TCR- and cytokine-dependent pathways to MAIT cell alterations in AH is unknown. Ex vivo studies showed that neither ALD plasma, which contained higher levels of bacterial products and inflammatory cytokines, nor alcohol treatment could induce MAIT cell depletion from PBMCs obtained from healthy donors (37). We also confirmed that plasma from neither patients with AH nor HDC could induce MAIT cell depletion from PBMCs of healthy donors. Based on our results and published studies (35,37), we propose that both antigen-dependent and cytokine-dependent pathways play a role in driving MAIT cell hyperactivation and depletion in patients with AH (Figure 5). Future study on the potential role of alcohol or alcohol metabolites, bacterial metabolites, and inflammatory cytokines in MAIT cell depletion in patients with AH is warranted.

HDC in our study were heavy drinkers without obvious signs of clinical liver disease, although their AST and ALT levels were slightly higher than HC. Our research groups have shown that those subjects have higher serum levels of LPS and markers of monocyte/macrophage activation (sCD14 and sCD163) compared to nonexcessive drinkers in terms of bacterial translocation (38). We showed here that HDC had a significant decrease of MAIT cells in the peripheral blood compared to HC. HDC had slightly increased levels of the bacterial translocation marker sCD14 and MAIT activation-associated cytokines IL-18 and IL-12. This increase was significant when 2-group comparison between HDC and HC was performed. However, this difference did not reach significance due to lower statistical power for 3-group comparison (HC, AH, and HDC). Our study indicates that chronic excessive drinking leads to slight immune abnormalities in HDC (17). Interestingly, MAIT cell frequency did not differ between HDC who stopped drinking and those who continued drinking at 12-month follow-up (data not shown). This MAIT cell stability is also reflected by the nonprogression of clinical disease severity. We have recently reported that the circulating microbiome in HDC is also altered compared to nondrinking controls (9), which might contribute to this MAIT cell abnormality in HDC.

Complete alcohol abstinence is the key to immune system recovery from AH, but it does not lead to full recovery in all patients (46). Consistent with this, clinical scores and liver function in our abstinent patients with AH greatly improved but were still abnormal during the follow-up (Supplementary Table 1, Supplementary Digital Content 3, http://links.lww.com/CTG/A54). We have recently reported that levels of several proinflammatory cytokines, such as TNF-α and IL-8, are still elevated in insentient patients with AH than HDC at the end of
In conclusion, we found that HDC had a reduction of MAIT cells despite showing little evidence of immune activation, whereas patients with AH had a severe depletion of MAIT cells and the residual cells had highly dysregulated expression of multiple T-cell activation markers in the background of a higher level of bacterial translocation and immune activation. MAIT cell dysfunction might be a contributing factor to the weakened immunity against microbial infection in patients with AH. Complete abstinence from alcohol consumption greatly but not completely reversed MAIT abnormalities. Current medical therapies for AH are limited. Thus, development of therapies to target dysregulated immune systems, including MAIT cells, may represent a promising strategy for restoring immune homeostasis and host defense against infections in AH.

CONFLICTS OF INTEREST
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Specific author contributions: W.L.: designed and performed all the experiments, performed statistical analysis and data analysis, interpreted data, and wrote the manuscript. E.L.L. and S.C.: did data analysis. J.L.: did experiments and artwork in Figure 5. S.R.: conducted experiments. S.I., P.P., P.S.K., A.J.S., V.H.S., S.R., D.W.C., and N.C.: provided clinical samples, funding and feedback for the project, and critically reviewed the manuscript. Q.Y.: conceptualized the study, obtained funding for the study, critically reviewed, and finalized the manuscript.
Financial support: This work was supported by the TREAT consortium that was created with the support of the National Institute on Alcohol Abuse and Alcoholism (NIAAA U01 AA021840, Translational Research and Evolving Alcoholic Hepatitis Treatment). Indiana University (NIAAA AA021883), Mayo Clinic (NIAAA AA021788), and Virginia Commonwealth University (NIAAA AA021891) are members of the TREAT consortium. This work was also supported by NIAAA grant (UH2AA026218 to Q.Y.).
Potential competing interests: None.

ACKNOWLEDGEMENTS
We thank all volunteers for donating their time and blood for this multicenter prospective observational cohort study. We thank Kayla Peterson for coordinating the study.

REFERENCES
1. Chayanupatkul M, Liangpunsakul S. Alcoholic hepatitis: A comprehensive review of pathogenesis and treatment. World J Gastroenterol 2014;20:6279–86.
2. Saberi B, Dadabhai AS, Jang YY, et al. Current management of alcoholic hepatitis and future therapies. J Clin Transl Hepatol 2016;4:113–22.
3. Fung P, Pyrsopoulos N. Emerging concepts in alcoholic hepatitis. World J Hepatol 2017;9:567–85.
4. Chan C, Levitsky J. Infection and alcoholic liver disease. Clin Liver Dis 2016;20:595–606.
5. Pasala S, Barr T, Messaoudi I. Impact of alcohol abuse on the adaptive immune system. Alcohol Res 2015;37:185–97.
6. Gao B, Seki E, Brenner DA, et al. Innate immunity in alcoholic liver disease. Am J Physiol Gastrointest Liver Physiol 2011;300:G516–525.
7. Nagy LE. The role of innate immunity in alcoholic liver disease. Alcohol Res 2015;37:237–50.
8. Mutlu EA, Gillevet PM, Rangwala H, et al. Colonic microbiome is altered in alcoholism. Am J Physiol Gastrointest Liver Physiol 2012;302:G966–978.
9. Puri P, Liangpunsakul S, Christensen JE, et al. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. Hepatology 2018;67:1284–302.
10. Leclercq S, Matamoros S, Cani PD, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A 2014;111:E4485–93.
11. Rao R. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. Hepatology 2009;50:638–44.
12. Gao B, Bataller R. Alcoholic liver disease: Pathogenesis and new therapeutic targets. Gastroenterology 2011;141:1572–85.
13. Jampana SC, Khan R. Pathogenesis of alcoholic hepatitis: Role of inflammatory signaling and oxidative stress. World J Hepatol 2011;3:114–7.
14. Degre D, Lemmers A, Gustot T, et al. Hepatic expression of CCL2 in alcoholic liver disease is associated with disease severity and neutrophil infiltrates. Clin Exp Immunol 2012;169:302–10.

15. Dhanda AD, Collins PL. Immune dysfunction in acute alcoholic hepatitis. World J Gastroenterol 2015;21:11904–13.

16. Markwick LJ, Riva A, Ryan JM, et al. Blockade of PD1 and TIM3 restores innate and adaptive immunity in patients with acute alcoholic hepatitis. Gastroenterology 2015;148:S90–602.e10.

17. Li W, Amet T, Xing Y, et al. Alcohol abstinence ameliorates the dysregulated immune profiles in patients with alcoholic hepatitis: A prospective observational study. Hepatology 2017;66:575–90.

18. Wong EB, Ndung’u T, Kasprzowicz VO. The role of mucosal-associated invariant T cells in infectious diseases. Immunology 2017;150:45–54.

19. Kurioka A, Walker LJ, Klenerman P, et al. MAIT cells: New guardians of intestinal immunity with antibacterial immune defects in alcoholic liver disease. Gut 2018;67:918–30.

20. Li et al. The role of mucosal-associated invariant T cells in infectious diseases. Immunology 2017;150:45–54.

21. Eccle SB, Corbett AJ, Keller AN, et al. Recognition of vitamin B precursors and byproducts by mucosal associated invariant T cells. J Biol Chem 2015;290:30204–11.

22. Kjer-Nielsen L, Corbett AJ, Chen Z, et al. An overview on the identification of MAIT cell antigens. Immunol Cell Biol 2018;96:573–87.

23. Ussher JE, Bilton M, Attwood E, et al. CD161 (+) TCR iValpha7.2(+) mucosal-associated invariant T cells: A novel population in chronic hepatitis B virus infection. Front Immunol 2015;6:041.

24. Yong YK, Saeidi A, Tan HY, et al. Hyper-expression of PD-1 is associated with the levels of exhausted and dysfunctional phenotypes of circulating CD161(+) TCR iValpha7.2(+) mucosal-associated invariant T cells in chronic hepatitis B virus infection. Front Immunol 2018;9:472.

25. Jeffery HC, van Wilgenburg B, Kurioka A, et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. J Hepatol 2016;64:1118–27.

26. Bottcher K, Rombouts K, Saffiotti F, et al. MAIT cells are chronically activated in patients with autoimmune liver disease and promote profibrogenic hepatic stellate cell activation. Hepatology 2018;68:172–186.

27. Riva A, Patel V, Kurioka A, et al. Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. Gut 2018;67:918–30.

28. Liangpunsakul S, Toh E, Ross RA, et al. Quantity of alcohol drinking positively correlates with serum levels of endotoxin and markers of monocyte activation. Sci Rep 2017;7:4462.

29. Liangpunsakul S, Purp P, Shah VH, et al. Effects of age, sex, body weight, and quantity of alcohol consumption on occurrence and severity of alcoholic hepatitis. Clin Gastroenterol Hepatol 2016;14:1831–8.e3.

30. Reantragoon R, Corbett AJ, Sakala IG, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. J Exp Med 2013;210:2305–20.

31. Hege P, Weiss E, Paradis V, et al. Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver. Nat Commun 2018;9:2146.

32. Gerard S, Siberil S, Martin E, et al. Human iNKT and MAIT cells exhibit a PLZF-dependent proapoptotic propensity that is counterbalanced by XIAP. Blood 2013;121:614–23.

33. Patel S, Behara R, Swanson GR, et al. Alcohol and the intestine. Biomolecules 2015;5:525–38.

34. O’Shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease. Hepatology 2010;51:307–28.

35. Cosgrove C, Ussher JE, Rauch A, et al. Early and nonreversible decrease of CD161(+) TCR iValpha7.2(+) MAIT cells in HIV infection. Blood 2013;121:951–61.

36. Leensah Y, Ganesh A, Quigley MF, et al. Mucosal-associated invariant T cells promote inflammation and exacerbate disease in murine models of arthritis. Arthritis Rheum 2012;64:153–61.

37. Loh L, Wang Z, Sant S, 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;113:10133–8.

38. Tang XZ, Jo J, Tan AT, et al. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. J Immunol 2013;190:3142–52.

39. Leensah Y, Svard J, Dias J, 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.

40. Jiang J, Yang B, An H, et al. Mucosal-associated invariant T cells from patients with tuberculosis exhibit impaired immune response. J Infect 2016;72:338–52.

41. Bolte FJ, Reherrmann B. Mucosal-associated invariant T cells in chronic inflammatory liver disease. Semin Liver Dis 2018;38:60–5.

42. Hengst J, Strunz B, Deterding K, et al. Nonreversible MAIT cell dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy. Eur J Immunol 2016;46:2204–10.

43. Yong YK, Saeidi A, Tan HY, et al. Hyper-expression of PD-1 is associated with the levels of exhausted and dysfunctional phenotypes of circulating CD161(+) TCR iValpha7.2(+) mucosal-associated invariant T cells in chronic hepatitis B virus infection. Front Immunol 2018;9:472.