MAIT Cells Are Enriched and Highly Functional in Ascites of Patients With Decompensated Liver Cirrhosis

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BACKGROUND AND AIMS: Patients with advanced liver cirrhosis have an increased susceptibility to infections. As part of the cirrhosis-associated immune dysfunction, mucosal-associated invariant T (MAIT) cells, which have the capacity to respond to bacteria, are severely diminished in circulation and liver tissue. However, MAIT cell presence and function in the peritoneal cavity, a common anatomical site for infections in cirrhosis, remain elusive. In this study, we deliver a comprehensive investigation of the immune compartment present in ascites of patients with decompensated liver cirrhosis, and focus especially on MAIT cells.

APPROACH AND RESULTS: To study this, matched peripheral blood and ascites fluid were collected from 35 patients with decompensated cirrhosis, with or without spontaneous bacterial peritonitis (SBP). MAIT cell phenotype and function were analyzed using high-dimensional flow cytometry, and the obtained data were compared with the blood samples of healthy controls (n = 24) and patients with compensated cirrhosis (n = 11). We found circulating MAIT cells to be severely decreased in patients with cirrhosis as compared with controls. In contrast, in ascites fluid, MAIT cells were significantly increased together with CD14+CD16+ monocytes, innate lymphoid cells, and natural killer cells. This was paralleled by elevated levels of several pro-inflammatory cytokines and chemokines in ascites fluid as compared with plasma. Peritoneal MAIT cells displayed an activated tissue-resident phenotype, and this was corroborated by increased functional responses following stimulation with E. coli or interleukin (IL)-12 + IL-18 as compared with circulating MAIT cells. During SBP, peritoneal MAIT cell frequencies increased most among all major immune cell subsets, suggestive of active homing of MAIT cells to the site of infection.

CONCLUSIONS: Despite severely diminished MAIT cell numbers and impaired phenotype in circulation, peritoneal MAIT cells remain abundant, activated, and highly functional in decompensated cirrhosis and are further enriched in SBP. This suggests that peritoneal MAIT cells could be of interest for immune-intervention strategies in patients with decompensated liver cirrhosis and SBP. (HEPATOLOGY 2020;72:1378-1393).

Liver cirrhosis is the end stage of many chronic liver diseases such as chronic viral hepatitis and alcohol-associated liver disease. Complications of cirrhosis are ascites, hepatic encephalopathy, esophageal varices bleeding, and/or occurrence of icterus, which define decompensated cirrhosis. Patients with decompensated cirrhosis have a 1-year cumulative mortality of 40%-50%. One of the main causes for acute decompensation with subsequent acute-on-chronic liver failure and death is infection, such as...
spontaneous bacterial peritonitis (SBP). One reason for the vulnerability for infections is the impaired immune defense of patients with advanced liver cirrhosis, known as cirrhosis-associated immune deficiency syndrome. This involves affected functions of serum proteins, including reduced capacity for opsonization, reduced levels of complement factors, and diminished protein C activity. Myeloid cells are affected by neutrophil and macrophage dysfunction and lower capacity of monocytes and dendritic cells to execute appropriate pro-inflammatory immune responses. However, less is known regarding the function of lymphocytes in liver cirrhosis.

One lymphocyte subset that is reduced in patients with liver cirrhosis are mucosa-associated invariant T (MAIT) cells. MAIT cells are innate-like T cells defined by the co-expression of the semi-invariant T-cell receptor (TCR) Vα7.2 chain and CD161. MAIT cells are present in peripheral blood but enriched in mucosal tissues and highly abundant in the liver. In addition, they are able to recognize riboflavin metabolites of bacterial or fungal origin presented by the major histocompatibility complex class I-like molecule MR1. Together with high expression of interleukin (IL)-12 and IL-18 receptors, MAIT cells have the capacity to interact with antigen-presenting cells and participate in immune responses toward pathogens. Following activation, they rapidly release cytokines such as tumor necrosis factor (TNF), interferon γ (IFNγ), and IL-17 and can lyse target cells. Because of their ability to recognize and respond to microbial metabolites, MAIT cells are critical in the response against bacteria.

Both the circulating and the intrahepatic MAIT cell compartment are severely impaired in patients with liver cirrhosis. Although not completely understood, this cirrhosis-associated immune dysfunction is likely a consequence of ongoing intrahepatic inflammatory processes that are hallmarks of chronic liver disorders. For instance, MAIT cells are impaired in patients with chronic hepatitis C, and elimination of hepatitis C virus by direct acting antivirals had no or only limited effects on restoring the MAIT cell compartment. Furthermore, with declining liver function, the amounts of microbial products that pass by the liver increases. This constant exposure to heightened levels of microbial products is likely also contributing to the loss of MAIT cells in liver cirrhosis. On the other hand, it has also been suggested that MAIT cells exhibit a pro-fibrogenic role through production of IL-17. However, so far, the role of MAIT cells in patients with decompensated cirrhosis and ascites, who are especially vulnerable to infections, have not been studied extensively. Although the most common cause of death in patients with advanced liver cirrhosis is infections, including SBP, urinary tract infections and pneumonia, little is known regarding local immune responses at these anatomical sites.
In this study, we deliver a comprehensive investigation of the immune compartment present in ascites of patients with decompensated liver cirrhosis, and focus especially on MAIT cells. In contrast to the severe impairment noted in circulation and liver, we report that the peritoneal MAIT cell compartment is largely intact with a robust capacity to respond to pathogens.

Materials and Methods

PATIENTS

Patients with decompensated cirrhosis and ascites were recruited from the prospective INFekta registry (DRKS00010664). Patients with compensated cirrhosis were seen at the Department of Gastroenterology, Hepatology and Endocrinology at Hannover Medical School. Written, informed consent was obtained from all participants, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethics Committee of Hannover Medical School (3188-2016). From patients with decompensated liver cirrhosis, matched blood (peripheral blood mononuclear cells [PBMCs] and plasma) and ascites (mononuclear cells [MNCs]) and supernatant) samples were collected. From patients with compensated cirrhosis, only blood (PBMCs and plasma) samples were collected. Additionally, paired ascites and blood samples were collected from patients with SBP defined according to the German Guidelines. Hepatocellular carcinoma and human immunodeficiency virus co-infection were exclusion criteria. Detailed patient characteristics are given in Supporting Table S1.

ISOLATION AND STORAGE OF PBMC, PLASMA, MONONUCLEAR CELLS FROM ASCITES, AND ASCITES SUPERNATANT

PBMCs were isolated from fresh whole blood using Ficoll-density gradient centrifugation and were cryopreserved in liquid nitrogen. Plasma was collected from ethylene diamine tetraacetic acid (EDTA) blood and stored at −80°C. Ascites samples were collected during paracentesis, and MNCs were isolated by centrifugation (5 minutes at 800 g) and lysis of red blood cells, followed by cryopreservation in liquid nitrogen. Aliquots of ascites supernatant were stored at −80°C until further use.

FLOW CYTOMETRY

Cryopreserved PBMCs and ascites MNCs were thawed, and fluorescence-activated cell sorting (FACS) staining with fluorochrome-labeled monoclonal antibodies was performed as previously described. For dead cell exclusion, all samples were stained either with fixable viability stain 700, Live Dead Fixable Aqua, or Live Dead Fixable Green. For fixation, an eBioscience Fixative (FOXP3/Transcription Buffer Staining Set; San Diego, CA) was used. The antibodies used are listed in Supporting Table S2. Samples were acquired either on a 16-color or 18-color LSRFortessa flow cytometer (BD Biosciences), and data were analyzed using FlowJo software v10.5.3. The MR1 tetramer technology was developed jointly by Dr. James McCluskey, Dr. Jamie Rossjohn, and Dr. David Fairlie, and the material was produced by the National Institutes of Health Tetramer Core Facility, as permitted to be distributed by the University of Melbourne. MAIT cells were identified as single, alive CD14−CD19−CD3+CD4−CD161hiVα7.2+ cells (Supporting Fig. S1). For high-dimensional analysis of FACS data, equal numbers of events of the respective samples were exported, barcoded to trace back origin, and concatenated prior to downstream analysis. Next, Uniform Manifold Approximation and Projection (UMAP) or PhenoGraph were performed with publicly available FlowJo plugins and the barcoded origin overlaid on either analysis results. PhenoGraph clusters were then ordered and analyzed accordingly.

MAIT CELL IN VITRO STIMULATION

PBMCs and ascites MNCs were stimulated with either IL-12 + IL-18 or Escherichia coli (E. coli DH5α) + α-CD28 (1.25 μg/mL) for 24 hours as previously described. In brief, PBMCs and ascites MNCs were stimulated with E. coli (5 or 10 bacteria per cell [BPC]) or IL-12 (10 ng/mL; Miltenyi Biotech, Bergisch Gladbach, Germany) + IL-18 (100 ng/mL; MBL International Corporation, Woburn, MA) to induce cytokine response. Phorbol 12-myristate 13-acetate/ionomycin (2 μg/mL; 500 ng/mL) was used
as a positive control. For the last 6 hours of stimulation, Brefeldin A (2 µg/mL; Golgi Plug; BD Biosciences) and monensin (Golgi Stop; BD Biosciences) were added. To assess degranulation, CD107a was added at the same time as brefeldin A and monensin. In selected experiments, MR-1 blocking antibody (10 µg/mL; clone 26.5; BioLegend, San Diego, CA) and appropriate IgG2a isotype control (clone MOPC-173; BioLegend) were added to E. coli–mediated stimulation assays at the beginning of the assay. In selected experiments, myeloid cells were isolated from either peripheral blood or ascites with the Pan Monocyte Isolation Kit (Miltenyi). To these, TCRVα7.2-labeled and anti-phycoerythrin (PE) microbead–enriched peritoneal MAIT cells were added as indicated. Subsequent functional experiments using isolated myeloid cells and MAIT cells were performed as described previously.

**CYTOKINE ASSAYS**

Cytokine levels of plasma and ascites supernatant were measured using the LumineX-based multiplex bead assay (Human Cytokine Assay; 12007283; BioRad Laboratories, Hercules, CA) according to the manufacturer’s instructions and optimized protocols. All samples were analyzed in one run and acquired using BioPlex Manager 6.0 software. Cytokine values that were below the detection range were determined as the lowest possible calculated concentration divided by two. Plasma and ascites supernatant levels of soluble CD14 (sCD14) and soluble CD163 (sCD163) were investigated using enzyme-linked immunosorbent assay (catalog numbers DC140 and DC1630, respectively; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**STATISTICAL ANALYSIS**

Statistical analyses were performed using GraphPad Prism software 8.0. First, the distribution of data sets was assessed by the D’Agostino & Pearson normality test. For normally distributed data, the unpaired t test was used to compare the two data sets. Matched samples were analyzed either with Wilcoxon matched-pairs signed rank test or paired t test, depending on the normal distribution of data or not. For multiple comparison, Kruskal-Wallis, Mann-Whitney U test, or one-way analysis of variance were used when indicated. Correlations between data sets were analyzed using either Pearson’s r or Spearman’s r coefficients. Details on the statistical tests are displayed in the respective figure legends, and for all graphs the significances are shown as *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

**Results**

**CIRCULATING MAIT CELLS ARE DECREASED IN CIRRHOSIS AND DISPLAY AN ACTIVATED PHENOTYPE**

We determined the frequency of blood MAIT cells, identified as CD4⁺CD161⁺TCRVα7.2⁺ T cells, in our cohort of patients with liver cirrhosis. Accordingly, using the MR1-tetramer, we demonstrate that our gating strategy to identify blood MAIT cells is valid (Supporting Fig. S1). Data were compared with healthy controls, and we stratified patients with cirrhosis based on different etiology and severity of disease for a subgroup analysis (Supporting Table S1). In line with previous reports, we observed a significantly lower frequency of MAIT cells in patients with cirrhosis compared with healthy controls (Fig. 1A). In contrast, within the cirrhosis cohort, no differences were noted between patients with compensated and decompensated cirrhosis (Supporting Fig. S2A). Furthermore, patients with alcohol-related liver cirrhosis (ARC) and patients with chronic viral hepatitis (HXV) showed no significant differences in MAIT cell frequencies (Supporting Fig. S2A). The MAIT cell loss in patients with liver cirrhosis equally affected the CD8⁺ and CD8⁻ MAIT cell subsets (Fig. 1A). With respect to clinical characteristics, MAIT cell frequency negatively correlated with liver stiffness, as measured by transient elastography and Model for End-Stage Liver Disease (MELD) score (Fig. 1B). We also noticed that the relative concentrations of sCD14 and sCD163, oblique markers of microbial translocation, were significantly higher in the plasma of patients with liver cirrhosis compared with healthy controls (Fig. 1C). Interestingly, sCD14 and sCD163 inversely correlated with MAIT cell percentages (Fig. 1D).

Next, we investigated the phenotype and function of blood MAIT cells in cirrhosis. MAIT cells from patients with cirrhosis were biased toward an activated phenotype, with higher levels of HLA-DR,
A

Healthy Cirrhosis

MAIT cells out of total T cells (%)

CD161

Va7.2

B

Fibroscan (kPa)

r = -0.556
p = 0.011

MAIT cells out of total T cells (%)

sCD14 plasma

sCD163 plasma

MELD - Score

r = -0.423
p = 0.035

r = -0.556
p = 0.011

C

sCD14 relative conc. (OD)

r = -0.364
p = 0.017

sCD163 relative conc. (OD)

r = -0.297
p = 0.043

D

E

Expression (%)

HLA-DR PD-1 Ki-67 CD56 CD16

CD25 CD28 CD38 CD127 CD57

F

IL-12 + IL-18 stimulation

E. coli 5 BPC stimulation
CD56, CD25, and CD38 and elevated proliferation indicated by Ki-67 (Fig. 1E). This activated phenotype is in line with previous reports. Of note, PD-1 expression was significantly higher in patients with compensated versus decompensated cirrhosis (Supporting Fig. S2B). MAIT cells from patients with HXV displayed higher PD-1 expression and were less proliferative than MAIT cells from patients with ARC (Supporting Fig. S2C). Following stimulation with IL-12 + IL-18 and \textit{E. coli} (5 BPC), MAIT cells from patients with cirrhosis \((n = 35, n = 25, \text{or } n = 10)\) compared to patients with liver cirrhosis \((n = 35)\). (D) Correlation of MAIT cell frequencies with sCD14 and sCD163 levels \((n = 10)\). (E) Phenotypic characterization of blood MAIT cells in healthy controls \((n = 24, n = 14, \text{or } n = 10)\) and patients with liver cirrhosis \((n = 35, n = 25, \text{or } n = 10)\). (F) Summary of functional MAIT cell responses following stimulation with IL-12 + IL-18 (left) and \textit{E. coli} (right); healthy controls \((n = 14)\) and patients with cirrhosis \((n = 14)\). Mann-Whitney U test and unpaired Student \(t\) test were used when appropriate, and correlations between clinical data and MAIT cell frequencies were assumed using Spearman's \(r\) for all nonparametric values. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\), and ****\(P < 0.0001\).

Taken together, these data show that MAIT cells are diminished in numbers in patients with liver cirrhosis while displaying an activated phenotype. These data are in line with previous reports and validate our cohort and analysis pipeline for the subsequent downstream analysis.

**IMMUNE ENVIRONMENT IN ASCITES FLUID OF PATIENTS WITH DECOMPENSATED CIRRHOSIS**

We hypothesized that the ascitic fluid in patients with decompensated cirrhosis represents a special immune environment, as its existence is linked to pathological processes. To address this, we applied a broad 21-parameter flow cytometry panel\(^{32}\) as well as a multiplex cytokine assay on ascites fluid samples. The obtained FACS data were analyzed using conventional gating, and UMAP\(^{28}\) was used to reduce dimensions and to identify main clusters of immune cells (Fig. 2A,B, Supporting Fig. S3). Only minor differences were noted when comparing the presence of different adaptive lymphocyte subsets in ascites fluid with peripheral blood (Fig. 2A-C, Supporting Fig. S3). Instead, a significant shift was observed within the monocyte population with a strong increase in CD14\(^+\)CD16\(^+\) intermediate monocytes and a reciprocal decline in classical and nonclassical monocytes (Fig. 2A-C). Furthermore, we noted significantly increased size and granularity for the CD14\(^+\)CD16\(^+\) subset in ascites when compared with similar cells in blood, suggesting that this population might be peritoneal macrophages (Supporting Fig. S3C).\(^{33}\) This was further corroborated by the higher surface expression of CD163 on these cells (Fig. 2B). Finally, innate lymphocytes such as innate lymphoid cells (ILCs) and different natural killer (NK) cell populations as well as MAIT cells were specifically increased in ascites fluid as compared with blood (Fig. 2A-C). Next, we addressed the soluble immune profile in patients with decompensated liver cirrhosis and compared ascites supernatants with plasma from the same patients (Fig. 2D). IL-7, important for MAIT cell survival and proliferation,\(^{34}\) was conspicuously higher in ascites, whereas IL-18, which has been suggested to promote MAIT cell apoptosis,\(^{35}\) was significantly lower. Moreover, IL-6, IL-10, IL-12p70, and IL-15 were more abundant in the ascites fluid, whereas IL-16 and RANTES (CCL5) were decreased.

In summary, ascites fluid from patients with decompensated cirrhosis display an immunological landscape skewed toward innate immune cells.

**MAIT CELLS ARE ENRICHED AND HIGHLY ACTIVATED IN ASCITES FLUID OF PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS**

As MAIT cells are among the more abundant cells present in ascites, we studied their role within the peritoneal cavity and stratified for the underlying etiology
of decompensation. Using the MR-1 tetramer, we validated that it is feasible to identify MAIT cells in ascites using CD161 and Vα7.2 together with excluding CD4+ T cells (Fig. 3A). In general, MAIT cell frequencies were significantly higher in ascites compared with matched blood samples (Fig. 3B). When stratifying for CD8 expression, we could identify that this increase was mostly due to a higher frequency of CD8+ MAIT cells. Furthermore, peritoneal MAIT cells were more frequent in patients with ARC as compared to patients with HXV (Fig. 3C,D). One hypothesis for the enrichment of MAIT cells in ascites compared with blood might be an altered threshold to undergo apoptosis. Indeed, peritoneal MAIT cells expressed lower levels of the pro-apoptotic molecule BAX (B cell lymphoma 2–associated X protein) (Supporting Fig. S4A), whereas no differences were noted with respect to Bcl-2.

Having established that MAIT cells were enriched in ascitic fluid as compared with blood, we next assessed the detailed phenotype of peritoneal MAIT cells. To this end, we simultaneously analyzed the expression of 12 surface receptors on MAIT cells and subjected these data to UMAP analysis followed by PhenoGraph (29) clustering of MAIT cells (Fig. 3E). Eleven phenotypic clusters could be identified when comparing peripheral

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**FIG. 2.** Characterization of the ascites fluid immune landscape. (A) UMAP analysis was performed on 12 surface markers for 10 matched ascites and blood samples with 5,000 alive CD45+ cells from each sample. UMAP plots from the ascites fluid of patients with decompensated cirrhosis and their respective blood samples displaying the major immune cell subsets (left) and a residual plot showing the differences in immune cell composition in ascites compared with blood (right). Single marker plots and identification of immune cell subsets are shown in Supporting Fig. S3. (B) A second UMAP analysis was performed on the gated monocyte/macrophage population displaying the main phenotypic clusters, their relative abundance in ascites compared with blood, and the markers included in the analysis. (C) Fold change (mean ± SEM) for the indicated immune cell subset frequencies between matched ascites and blood samples from patients with decompensated cirrhosis (n = 10). (D) Median fold change for the indicated cytokines between matched ascites and blood samples from patients with decompensated cirrhosis (n = 10).
FIG. 3. MAIT cells are more abundant in ascites fluid and display a highly activated phenotype. (A) Representative FACS plots from one matched blood and ascites sample out of the five investigated, showing MR1 5-OP-RU tetramer staining within the Vα7.2+CD161+ T-cell subset divided based on CD4 and CD8 expression. (B) MAIT cell frequency out of the total T cells in matched blood and ascites as well as the distribution of the CD8+ and CD8−CD4− (double negative [DN]) subset (n = 24). (C) FACS plots showing representative stainings for MAIT cells in ascites fluid of 1 patient with ARC compared to 1 patient with HXV. (D) Peritoneal MAIT cell frequencies out of the total T cells as well as the distribution of the CD8+ and CD8−CD4− (DN) subset in patients with ARC (n = 18) and HXV (n = 9). (E) UMAP analysis and PhenoGraph clustering on MAIT cells from paired ascites and blood samples (n = 10 pairs), displaying the relative abundance of each group, the identified phenotypic clusters through PhenoGraph analysis, and UMAP plots for each of the 12 markers that were included in the analysis. (F) Relative abundance of each of the identified PhenoGraph clusters comparing blood and ascites (upper panel), and the deconvoluted phenotype of each cluster displayed as a heatmap (lower panel).
blood and peritoneal MAIT cells, with several clusters showing a higher relative abundance for ascites or peripheral blood origin (Fig. 3F). Interestingly, a higher expression of CD69, indicating tissue residency, was found in clusters related to peritoneal MAIT cells when compared with peripheral blood (Fig. 3F). In more detail, the defining elements for the three most prevalent clusters in peritoneal MAIT cells were either loss of CD28 (cluster 1), expression of tissue residency markers (CD69 and CD103, cluster 2), or combined CD69 and CD38 expression (cluster 3, Fig. 3F). The observed phenotypic differences from the dimensionality reduction analysis could be recapitulated using conventional FACS analysis, and some additional differences were observed (Supporting Fig. S4B,C).

Despite their activated phenotype, we observed lower levels of transcription factors T-bet and Eomes in peritoneal MAIT cells, but a larger fraction of these displayed an effector memory phenotype (Supporting Fig. S4C). Of note, CXCR3 and CXCR4 expression on peritoneal MAIT cells were significantly higher compared with blood MAIT cells (Supporting Fig. S4D). In the subgroup analysis of peritoneal MAIT cells from patients with ARC and HXV, only minor differences were observed (Supporting Fig. S5A).

To conclude, these results indicate that MAIT cells are more abundant in ascites as compared with peripheral blood in patients with decompensated liver cirrhosis and that peritoneal MAIT cells display a highly activated phenotype.

TISSUE-RESIDENT PERITONEAL MAIT CELLS PRESENT WITH A UNIQUE PHENOTYPE

Next, we studied the tissue residency profile of MAIT cells in different conditions in more detail. When comparing blood from patients with cirrhosis to blood from healthy donors, we interestingly observed higher frequencies of MAIT cells expressing the putative residency markers CD69 and CD103 in patients with cirrhosis (Fig. 4A,B). When assessing peritoneal MAIT cells in patients with decompensated cirrhosis, we observed an even higher frequency of these expressed CD69, CD103, and CD49a as compared with circulating MAIT cells (Fig. 4A,C).

We next investigated whether seemingly tissue-resident and non-tissue-resident peritoneal MAIT cells differed in their phenotype. This analysis revealed that the tissue-resident peritoneal MAIT cells expressed higher levels of PD-1 and CD38 but lower levels of CD127 and CD28 as compared with the non-tissue-resident MAIT cells (Fig. 4D). Thus, some of the phenotypic differences we observed when comparing circulating and total peritoneal MAIT cells (Fig. 3) could be ascribed to the unique phenotype of the tissue-resident peritoneal MAIT cells.

PERITONEAL MAIT CELLS FROM PATIENTS WITH DECOMPENSATED LIVER DISEASE ARE HIGHLY FUNCTIONAL

We next assessed the functional capacity of peritoneal MAIT cells following bacterial and innate cytokine stimulation (Fig. 5A). Strikingly, after IL-12 + IL-18 stimulation, peritoneal MAIT cells from patients with decompensated cirrhosis produced significantly higher levels of IFNγ and granzyme B and degranulated more as compared with matched blood MAIT cells (Fig. 5B). Similarly, significantly higher levels of IFNγ, TNF, and granzyme B were produced from peritoneal MAIT cells when stimulated with E. coli (Fig. 5C). Comparing purified peripheral blood and ascites-derived myeloid cells, we observed similar potential of these to activate ascites-derived MAIT cells (Supporting Fig. S6A,B). Along the same lines, the peritoneal MAIT cell response towards E. coli was also predominantly MR-1-dependent, as it could be strongly reduced using an MR-1 blocking antibody (Supporting Fig. S6C). Finally, we correlated the cytokine profiles from patients with decompensated liver cirrhosis with the in vitro functionality of MAIT cells. Surprisingly, IL-18 levels negatively correlated with MAIT cell function following innate cytokine stimulation (Fig. 5D).

Taken together, this shows that peritoneal MAIT cells are highly functional to both bacterial and cytokine stimulation.

MAIT CELLS ARE ENRICHED AND MANIFEST A STABLE PHENOTYPE IN ASCITES FROM PATIENTS DEVELOPING SPONTANEOUS BACTERIAL PERITONITIS

Finally, we aimed to investigate the impact of ongoing SBP on the frequency and phenotype of peritoneal MAIT cells and the cytokine milieu within
the peritoneal cavity. Patients with SBP displayed both significantly higher MAIT cell frequencies and total numbers in ascites fluid compared with noninfectious patients (Fig. 6A,B). Compared with other major immune cell subsets in ascites, MAIT cells were indeed the most enriched population in SBP compared with non-SBP (Fig. 6C). Surprisingly, despite the striking increase in frequency and the ongoing bacterial infection in ascites, there were no major differences in the phenotype of peritoneal MAIT cells between patients with and without SBP after UMAP and PhenoGraph analysis (Fig. 6D, Supporting Fig. S5B). Yet, we noted a trend in SBP-related clusters, in which MAIT cells less frequently expressed tissue residency markers (Fig. 6E). Furthermore, peritoneal MAIT cells in SBP showed no signs of ongoing proliferation (Supporting Fig. S5B). Consistent with these data, we found only minor differences in ascites cytokine profiles between SBP and non-SBP, with higher levels of CCL5 (RANTES), IL-1RA, and migration inhibitory factor in patients developing SBP, whereas IL-7 was among one of the most reduced cytokines in SBP ascites (Fig. 6F).

Altogether, this suggests that MAIT cells are actively recruited to the peritoneal cavity during SBP.

**Discussion**

Our work provides a comprehensive analysis of the ascites fluid immune landscape and a detailed characterization of antimicrobial MAIT cells in this
FIG. 5. Peritoneal MAIT cells display enhanced function in patients with decompensated cirrhosis. (A) Representative concatenated FACS plots showing peritoneal MAIT cell responses after stimulation with IL-12 + IL-18 or *E. coli* compared with unstimulated medium controls. Summary of functional responses following IL-12 + IL-18 (B) or *E. coli* stimulation (C). Indicated markers were analyzed on matched blood and ascites samples from patients with decompensated cirrhosis (*n* = 11). (D) Correlation analysis between MAIT cell functional responses after IL-12 + IL-18 stimulation and IL-18 levels. Function of blood and ascites samples were compared using the paired *t* test or Wilcoxon test when appropriate. *P* < 0.05, **P** < 0.01, and ***P** < 0.001. Spearman’s *r* was used to correlate among nonparametric data.
FIG. 6. MAIT cells are recruited to the peritoneal cavity during spontaneous bacterial peritonitis. (A) MAIT cell frequencies out of the total T cells and total number of MAIT cells per milliliter of ascites in patients with decompensated cirrhosis (no SBP, n = 27) compared to decompensated cirrhosis with ongoing SBP (SBP, n = 11). (B) Frequency of CD8+ and DN subsets of MAIT cells out of the total T cells in patients with SBP compared to patients without bacterial infection. (C) Ratio of immune cell subset frequencies in ascites fluid from patients with SBP compared to patients without SBP. (D,E) UMAP analysis and PhenoGraph clustering of MAIT cells from patients with decompensated cirrhosis (n = 10) and patients with ongoing SBP (n = 8). Origin of immune cells displayed in UMAP analysis (D) and relative abundance of clusters as well as respective phenotype after PhenoGraph analysis (E). Single parameter UMAP plots can be found in Supporting Fig. S5B. (F) Fold change for indicated cytokines between ascites from patients with SBP (n = 10) compared to patients without SBP (n = 10). Comparisons between groups were performed using the Mann-Whitney U test for nonparametric data sets. *P < 0.05 and **P < 0.01.
compartment in patients with decompensated liver cirrhosis. Our data yield insights into the underlying immunology of end-stage liver disease. In more detail, we unveil an enrichment of MAIT cells in the peritoneal cavity and show that they are highly activated and functional, whereas circulating MAIT cells are dramatically decreased in patients with cirrhosis.\(^{(6,7)}\) In fact, circulating MAIT cell frequencies exhibited a negative correlation with MELD score, suggesting a progressive loss of MAIT cells with decreasing liver function. We further report that circulating MAIT cells also correlate with plasma levels of sCD163 and sCD14, markers of monocyte/macrophage activation often downstream to microbial translocation\(^{(35)}\) indicating that MAIT cell depletion might be a direct consequence of contact with gut bacteria.\(^{(22)}\) Translocation of gut bacteria increases with portal hypertension,\(^{(21)}\) and this could explain why we observed a further decrease of MAIT cells with increased liver stiffness, an indirect marker of portal hypertension.\(^{(36)}\) The observed increase of MAIT cells in ascites may be explained by recruitment from portal hypertension–affected organs: MAIT cells in tissue express tissue-residency markers such as CD69, CD103, and T-bet, despite their higher activation status as compared with circulating MAIT cells isolated from blood. The higher amount of peritoneal tissue–resident MAIT cells may also contribute to the lower expression of Eomes and T-bet, despite their higher activation status as compared with blood MAIT cells.\(^{(43-45)}\) Consistently, we found elevated levels of IL-7 and IL-15 in the ascitic fluid. These cytokines are known to activate tissue-resident memory cells as well as MAIT cells.\(^{(34,40)}\) We report that the immune milieu of the ascites is vastly different compared with peripheral blood and it might contribute to the activated phenotype and increased MAIT cell function observed. Interestingly, many antigen-presenting cells, such as B cells and myeloid dendritic cells as well as classical and nonclassical monocytes, were less abundant in ascites fluid as compared with blood. Instead, we show an approximate 7-fold increase in peritoneal CD14\(^+\)CD16\(^-\) intermediate monocytes. This is in line with previous studies,\(^{(33)}\) and because these myeloid cells were larger and more granular and expressed higher levels of CD163, they might represent peritoneal macrophages. We did not observe differential capacity to stimulate MAIT cells between peritoneal and peripheral blood-derived myeloid cells, so the increased peritoneal MAIT cell functionality might be due to the immune milieu of soluble factors and the global cell compartment present in ascites. Indeed, the peritoneal cavity cytokine milieu was also different as compared with plasma.
In line with previous reports, we found an increase of cytokines, which support monocytes/macrophages migration/differentiation (CCL7 [MCP-3], CCL2 [MCP-1], and GM-CSF)[46,47] as well as IL-7, which has been shown to support MAIT cell reactivity.[34,48]

One can speculate that the combination of immune cell composition and cytokine milieu primes peritoneal MAIT cells to respond more potently following innate cytokine stimulation.

During SBP, we observed both an increase in the relative frequency of MAIT cells among total T cells as well as a dramatic increase in absolute numbers of MAIT cells. Furthermore, among all immune cell subsets investigated, MAIT cells were the subset that was most enriched in SBP compared to patients without SBP. Interestingly, the phenotype of peritoneal MAIT cells remained stable in patients with SBP. In line with this, and compared with the substantial changes between blood and ascitic fluid, we also detected fewer differences in the cytokine and chemokine milieu between patients with and without SBP. Here is of interest the reduced level of IL-7 in SBP and despite this, increased numbers of MAIT cells. Because the ex vivo levels of proliferation were low, this increase in MAIT cells is most likely due to active recruitment of these cells to the peritoneal cavity, as eluded to previously. These data are also in line with a previous report on patients with noncirrhotic end-stage kidney disease undergoing peritoneal dialysis that evolves bacterial peritonitis.[49] and underlines the importance of MAIT cells in ascites and SBP.

In conclusion, we show that MAIT cells are enriched in the ascites of patients with decompensated liver cirrhosis and even more abundantly in SBP. Peritoneal MAIT cells display an activated tissue-resident phenotype and are highly functional toward both bacterial and innate cytokine stimulation. The higher functionality of peritoneal MAIT cells compared with circulating MAIT cells underlines the importance of this immune cell subset for patients with advanced cirrhosis. One possibility of using MAIT cells as potential targets for immune interventions could be to improve co-stimulatory pathways to support MAIT cell expansion and thereby enhance control of infection. In this regard, Wang et al. showed that combining IL-23 with 5-OP-RU (5-[2-oxopropylidenamino]-6-D-ribityl-aminouracil) augments MAIT cell-mediated control of pulmonary Legionella infection in mice,[50] and future studies should explore how MAIT cells could be used as vaccine targets in patients with cirrhosis-associated immune dysfunction.

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Author Contributions: C.N., A.K., N.B., and M. Cornberg were responsible for the study design. C.N., B.S., M. Cornillet, and C.F. were responsible for performing the experiments. C.N., B.S., M. Cornillet, A.S., A.K., N.B., and M. Cornberg were responsible for the data analysis. C.N., B.S., A.K., N.B., and M. Cornberg were responsible for the draft of the manuscript. B.M., S.H., and M.M. were responsible for the patient recruitment. All authors contributed to the critical revisions and approved the final manuscript.

REFERENCES

1) Tschantzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749-1761.
2) Nadim MK, Durand F, Kelhum JA, Levitsky J, O’Leary JG, Karvellas CJ, et al. Management of the critically ill patient with cirrhosis: a multidisciplinary perspective. J Hepatol 2016;64:717-735.
3) Hernaez R, Sola E, Moreau R, Gines P. Acute-on-chronic liver failure: an update. Gut 2017;66:541-553.
4) Jalan R, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, et al. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. J Hepatol 2014:60:1310-1324.
5) Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. J Hepatol 2014;61:1385-1396.
6) Bolte FJ, O’Keefe AC, Webb LM, Serti E, Rivera E, Liang TJ, et al. Intra-hepatic depletion of mucosal-associated invariant T cells in hepatitis C virus-induced liver inflammation. Gastroenterology 2017;153:1392-1403.e2.
7) Hegde P, Weiss E, Paradis V, Wan J, Mahire M, Sukriti S, et al. Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver. Nat Commun 2018;9:2146.
8) 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.
9) Fergusson JR, Huhn MH, Swadling L, Walker LJ, Kurioka A, Libbre A, et al. CD161(int)/CD8+ T cells: a novel population of highly functional, memory CD8+ T cells enriched within the gut. Mucosal Immunol 2016;9:401-413.
10) Dusseaux M, Martin E, Serriani 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.
11) Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MRI presents microbial vitamin B metabolites to MAIT cells. Nature 2012;491:717-723.
12) Xiao X, Cai J. Mucosal-associated invariant T cells: new insights into antigen recognition and activation. Front Immunol 2017;8:1540.
13) Huang S, Gillillan S, Cella M, Miley MJ, Lantz O, Lybarger L, et al. Evidence for MR1 antigen presentation to mucosal-associated invariant T cells. J Biol Chem 2005;280:21183-21193.
14) Ussher JE, Bilton M, Attwood 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.
15) Le Bourhis L, Martin E, Pegailllet I, Guihot A, Froux N, Core M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 2010;11:701-708.
16) Le Bourhis L, Dusseaux M, Bohineuert A, Bessoles S, Martin E, Premel V, et al. MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS Pathog 2013;9:e1003681.
17) Kurioka A, Walker IJ, Klerenner P, Willberg CB. MAIT cells: new guardians of the liver. Clin Transl Immunology 2018;7:201-207.
18) Ussher JE, Willberg CB, Klerenner P. MAIT cells and viruses. Hengst JH, et al. CD161++ CD8+ T cells, including the MAIT cell activation. Hepatology 2018;68:172-186.
19) Moghadamrad S, McCoy KD, Geuking MB, Sagesser H, et al. MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS Pathog 2013;9:e1003681.
20) Hengst J, Struzn B, Detering K, Ljunggren HG, Leeansyah E, Manns MP, et al. Nonreversible MAIT cell dysfunction in human hepatitis C virus infection despite successful interferon-free therapy. Eur J Immunol 2016;46:2204-2210.
21) Moghadamrad S, McCoy KD, Geuking MB, Sageserr H, Kirunid J, Macpherson AJ, et al. Attenuated portal hypertension in germ-free mice: function of bacterial flora on the development of mesenteric lymphatic and blood vessels. HEPATOLOGY 2015;61:1685-1695.
22) Riva A, Patel V, Kurioka A, Jeffery HC, Wright G, Tarff S, et al. Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. Gut 2018;67:918-930.
23) Arvaniti V, D’Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology 2010;139:1246-1256.e5.
24) Reuken PA, Stallmach A, Bruns T. Mortality after urinary tract infections in patients with advanced cirrhosis—relevance of acute kidney injury and comorbidities. Liver Int 2013;33:220-230.
25) Gerbes AL, Gubberg Y, Sauerbruch T, Wiest R, Appenrodt B, Bahr MJ, et al. [German S 3-guideline “ascites, spontaneous bacterial peritonitis, hepatoportal syndrome“]. Z Gastroenterol 2011;49:749-779.
26) Bjorkstrom NK, Fauriat C, Bryceson YT, Sandberg JK, Ljunggren HG, Malmberg KJ. Analysis of the KIR repertoire in human NK cells by flow cytometry. Methods Mol Biol 2010;612:353-364.
27) 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.
28) Becht E, McInnes IJ, Healy J, Dutearte CA, Kwock IWH, Ng LG, et al. Dimensionality reduction for visualizing single-cell data using UMAP. Nat Biotechnol 2019;37:38-44.
29) Levine JH, Simonds EF, Bendall SC, Davis KL, el Amir AD, Tadmor MD, et al. Data-driven phenotypic dissection of AML reveals progenitor-like cells that correlate with prognosis. Cell 2015;162:184-197.
30) Dias J, Sobkowiak MJ, Sandberg JK, Leeansyah E. Human MAIT-cell responses to Escherichia coli: activation, cytokine production, proliferation, and cytotoxicity. J Leukoc Biol 2016;100:233-240.
31) Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, et al. Stepwise development of MAIT cells in mouse and human. PLoS Biol 2009;7:e54.
32) Hengst J, Theorell J, Dettering K, Potthoff A, Dettmer A, Ljunggren HG, et al. High-resolution determination of human immune cell signatures from fine-needle liver aspirates. Eur J Immunol 2015;45:2154-2157.
33) Ruiz-Alcaraz AJ, Carmona-Martinez V, Tristan-Manzano M, Machado-Linde F, Sanchez-Ferrer ML, Garcia-Penarrubia P, et al. Characterization of human peritoneal monocyte/macrophage subsets in peritoneal: phenotype, GATA6, phagocytic/oxidative activities and cytokine expression. Sci Rep 2018;8:12794.
34) 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.
35) Dias J, Hengst J, Parrot T, Leeansyah E, Lunemann S, Malone DFG, et al. Chronic hepatitis delta virus infection leads to functional impairment and severe loss of MAIT cells. J Hepatol 2019;71:301-312.
36) Castera L, Pinzani M, Bosch J. Noninvasive evaluation of portal hypertension using transient elastography. J Hepatol 2012;56:696-703.
37) Vuillot V, Buggert M, Slichter CK, Berkson JD, Mair F, Addison MM, et al. Human MAIT cells exit peripheral tissues and recirculate via lymph in steady state conditions. JCI Insight 2018;3.
38) Sakala IG, Kjer-Nielsen L, Eickhoff CS, Wang X, Blazevic A, Liu L, et al. Functional heterogeneity and antimycobacterial effects of mouse mucosal-associated invariant T cells specific for riboflavin metabolites. J Immunol 2015;195:587-601.
39) Yasumoto K, Koizumi K, Kawashima A, Saitoh Y, Arita Y, Shinohara K, et al. Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. Cancer Res 2006;66:2181-2187.
40) Topham DJ, Reilly EC. Tissue-resident memory CD8+ T cells: from phenotype to function. Front Immunol 2018;9:515.
41) Selin LK, Cornberg M. Embedding T cells in the matrix. Nat Rev Immunol 2011;11:762-774.
42) Wang XZ, Stepp SE, Brehm MA, Chen HD, Selin LK, Welsh RM. Virus-specific CD8 T cells in peripheral tissues are more resistant to apoptosis than those in lymphoid organs. Immunity 2003;18:631-642.
43) Hombrink P, Helbig C, Backer RA, Piet B, Oja AE, Stark R, et al. Programs for the persistence, vigilance and control of human CD8(+) lung-resident memory T cells. Nat Immunol 2016;17:1467-1478.
44) Zhang N, Bevan MJ. Transforming growth factor-beta signaling controls the formation and maintenance of gut-resident memory T cells by regulating migration and retention. Immunity 2013;39:687-696.
45) Mackay LK, Wynne-Jones E, Freestone D, Pellicci DG, Mielke LA, Newman DM, et al. T-box transcription factors combine with the cytokines TGF-beta and IL-15 to control tissue-resident memory T cell fate. Immunity 2015;43:1101-1111.
46) Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nat Rev Immunol 2011;11:762-774.
47) Lacey DC, Achuthan A, Fleetwood AJ, Dinh H, Roiniotis J, Scholz GM, et al. Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. J Immunol 2012;188:5752-5765.
48) 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.
49) Liuzzi AR, Kift-Morgan A, Lopez-Anton M, Friberg IM, Zhang J, Brook AC, et al. Unconventional human T cells accumulate at the site of infection in response to microbial ligands and induce local tissue remodeling. J Immunol 2016;197:2195-2207.
50) Wang H, Kjer-Nielsen L, Shi M, D'Souza C, Pediongco TJ, Cao H, et al. IL-23 costimulates antigen-specific MAIT cell activation and enables vaccination against bacterial infection. Sci Immunol 2019;4:eaaw0402.

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