Research Article

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Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1

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Background & Aims: Mucosal-Associated Invariant T (MAIT) cells are innate-like T cells characterised by the invariant TCR-chain, Vα7.2-Jα33, and are restricted by MR1, which presents bacterial vitamin B metabolites. They are important for antibacterial immunity at mucosal sites; however, detailed characteristics of liver-infiltrating MAIT (LI-MAIT) and their role in biliary immune surveillance remain unexplored.

Methods: The phenotype and intrahepatic localisation of human LI-MAIT cells was examined in diseased and normal livers. MAIT cell activation in response to *E. coli*-exposed macrophages, biliary epithelial cells (BEC) and liver B cells was assessed with/without anti-MR1.

Results: Intrahepatic MAIT cells predominantly localised to bile ducts in the portal tracts. Consistent with this distribution, they expressed biliary tropic chemokine receptors CCR6, CXCR6, and integrin αEβ7. LI-MAIT cells were also present in the hepatic sinusoids and possessed tissue-homing chemokine receptor CXCR3 and integrins LFA-1 and VLA-4, suggesting their recruitment via hepatic sinusoids. LI-MAIT cells were enriched in the parenchyma of acute liver failure livers compared to chronic diseased livers. LI-MAIT cells had an activated, effector memory phenotype, expressed α4β7 and receptors for IL-12, IL-18, and IL-23. Importantly, in response to *E. coli*-exposed macrophages, liver B cells and BEC, MAIT cells upregulated IFN-γ and CD40 Ligand and degranulated in an MR1-dependent, cytokine-independent manner. In addition, diseased liver MAIT cells expressed T-bet and RORγt and the cytokines IFN-γ, TNF-α, and IL-17.

Conclusions: Our findings provide the first evidence of an immune surveillance effector response for MAIT cells towards BEC in human liver; thus they could be manipulated for treatment of biliary disease in the future.

Keywords: Human liver; Mucosal-associated invariant T cells; Biliary epithelium; *E. coli*; Immune response; Biliary firewall.

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Introduction

Mucosal-associated invariant T (MAIT) cells are a recently identified subset of T cells with an evolutionarily conserved invariant T cell antigen receptor (TCR) α-chain, composed of the invariant α-chain Vα7.2-Jα33/Jα20/Jα12 in humans and Vα19-Jα33 in mice [1,2]. They are restricted to the $CD161^{++}$ population and are abundant in human blood, the intestinal mucosa and mesenteric lymph nodes [3–5]. MAIT cells respond to antigen presented on the highly phylogenetically conserved major histocompatibility complex (MHC) class I-related molecule, MR1, which possesses a unique antigen-binding cleft for vitamin B metabolites from pathogenic and/or commensal bacteria, and distinguishes MAIT cells from peptide- or lipid-recognizing αβ T cells [1,6,7]. MAIT cells can be activated by a wide variety of bacterial strains *in vitro*, and importantly they are crucial in mucosal immune defence in bacterial infection [8–10]. They respond in an MR1-dependent manner to antigen presenting cells (APC) cultured with bacteria and can also be activated via IL-12 and IL-18 in a TCR-independent manner [11,12]. MAIT cell frequencies have been reported to be lower in bacterially-infected patients' blood [10,13].

Both hepatic sinusoids and biliary epithelial cells (BEC) are crucial in first-line defence towards pathogens in both the steady and disease state as the human liver is continuously exposed to intestinally-derived antigens from portal venous blood and biliary reflux [14]. A recent study suggested that immune cells in the

hepatic sinusoids function as a firewall to prevent the systemic spread of gut-derived pathogens that evade the mesenteric immune system [15]. The presence of MAIT cells has been reported in healthy human liver sinusoidal fluids [16], however, their role in mucosa defence at the bile ducts, which are continuous with the gut lumen and its microbes, and form the first-line protection against biliary pathogens, is still unexplored [17,18]. BEC are known to express antigen presenting molecules and can activate lymphocytes [19]. A recent report indicated that MAIT cells could efficiently lyse epithelial cells of the HeLa cell line that are infected with bacteria [20]. Taken together, these findings indicate that MAIT cells are likely to be important contributors to the maintenance of steady state immunity and the pathogenesis of inflammatory and biliary liver diseases, especially in response to bacterial exposure. Thus, in the current study, we used primary human liver tissues, obtained from both normal and diseased explanted human livers, to investigate the localisation and phenotype of intrahepa tic/liver-infiltrating MAIT (LI-MAIT) cells, as well as their functional response to bacterially-exposed biliary epithelial surfaces in inflammatory biliary liver diseases.

Materials and methods

Isolation of liver-infiltrating lymphocytes (LIL), peripheral blood lymphocytes (PBL), and BEC

Venous blood, collected in EDTA, was obtained from healthy donors, and patients with inflammatory and autoimmune liver diseases (primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC)) and alcoholic liver disease (ALD). Explanted diseased liver tissue was obtained from patients who underwent liver transplantation for end-stage liver diseases including PSC, PBC, ALD, and non-alcoholic steatohepatitis (NASH) or for acute liver failure from seronegative (NonA NonB (NANB)) hepatitis. Non-diseased liver tissues were obtained from unused donor liver tissues. All samples were collected with appropriate patient consent and local research ethics committee approval (LREC ref. CA/5192, 06/Q2708/11). Human LIL, PBL, and BEC cells were isolated from fresh liver tissue and peripheral blood as described previously [21].

Localisation of TCR Vα7.2-expressing cells

Human liver tissues were stained with purified anti-TCR Vα7.2 (50 μg/ml, 3C10, BioLegend) or IgG1 isotype control to detect the localisation of Vα7.2$^+$ cells. See Supplementary material.

Phenotyping of intrahepatic and peripheral blood MAIT cells

Liver-infiltrating and blood MAIT cells were phenotyped directly *ex vivo* for the expression of surface markers, transcription factors and intracellular cytokines. See Supplementary material.

MAIT cell response to E. coli-exposed antigen presenting cells

APCs: blood monocyte-derived macrophages, THP1, liver B cells or BEC were incubated overnight with paraformaldehyde-fixed *Escherichia coli* (*E. coli*) (DH5α, Invitrogen) at 25, 20, 1000, or 1000 bacteria per cell respectively. CD8$^+$ T cells isolated from blood using CD8 Microbeads (Miltenyi Biotec) or CD3$^+$ T cells isolated from liver by flow sorting were cultured with the *E. coli*-exposed APCs, in the presence of anti-CD107a (Pe or PeCy5) and blocking antibodies against IL-12p40/70 (5 μg/ml, C8.6, eBioscience), IL-18 (5 μg/ml, 125-2H, MBL International, USA) and MR1 (10 μg/ml) [22] as indicated. In some assays, anti-CD40 Ligand (CD40L)-PeCy7 was added. MAIT cell intracellular and surface markers were stained and data were acquired on a MACSQuant (Miltenyi Biotec) or CyAN (Dako) flow cytometer and analyzed using FlowJo (Tree Star Inc.). Autologous liver-infiltrating B cells and T cells were cell sorted by a Moflo Astrios cell sorter (Beckman Coulter). Cells were labeled with anti-CD3-PeCy7 and anti-CD19-APCVio770 to identify CD3$^+$ T cells and CD19$^+$ B cells respectively. Blood macrophages were generated by culturing CD14$^+$ monocytes, isolated from blood using CD14 microbeads (Mlltenyi Biotec), with 100 ng/ml M-CSF (R and D Systems) for 7–9 days.

Statistical analysis

GraphPad Prism 5.0 software (GraphPad software, San Diego, CA, USA) was used for statistical analysis. Comparisons of two populations were by the Mann-Whitney *U* test or *t* test. Comparisons of more than one population were by Friedman's test with Dunn's Multiple Comparison post-hoc test or by one-way ANOVA with Bonferroni's multiple comparison post hoc test as indicated in the figure legend. Statistical significance was defined as p value <0.05. Error bars on graphs are presented as median ± interquartile range or mean ± SEM. Values in text are given as median and overall range (in brackets).

Results

Intrahepatic MAIT cells preferentially reside in peri-biliary areas of portal tracts

We examined the localisation of LI-MAIT cells in normal and diseased human livers by immunohistochemistry staining for TCR Vα7.2. Most Vα7.2$^+$ cells resided around bile ducts in portal tracts with few detected in the parenchyma (Fig. 1A, B; Supplementary Fig. 2). The distribution was similar in normal, autoimmune, and non-autoimmune diseased livers (Fig. 1C; Supplementary Fig. 2) similar to other immune subsets (Supplementary Fig. 1). Interestingly, in acute, seronegative liver failure, increased infiltration of Vα7.2$^+$ cells to the parenchyma was noted (Fig. 1A iii, vi, 1C; Supplementary Fig. 3) when compared to normal livers or any of the chronic liver diseases studied (Fig. 1A i, iv). The overall frequency of Vα7.2$^+$ cells appeared increased in PSC compared to the other liver diseases (Fig. 1C). By flow cytometry, we showed that the majority of Vα7.2$^+$ lymphocytes in normal livers (63.6% (24.4–93.2%)) and over one-third in diseased (40.5% (11.6–75.2%)) were CD3$^+$CD161^{++} MAIT cells (Supplementary Fig. 4). We confirmed the predominant localisation of CD3$^+$ CD161$^+$ Vα7.2$^+$ MAIT cells in peri-biliary regions of portal tracts by both immunohistochemistry (Fig. 1Aii, v; 1C) and confocal microscopy (Fig. 2).

Frequencies of MAIT cells are reduced in liver diseases, with an increase in the CD4$^+$ MAIT cells

Next, using flow cytometry we compared frequencies of CD3$^+$ CD161^{++} Vα7.2$^+$ MAIT cells in intrahepatic liver infiltrates and in blood from normal and diseased tissues. Increased frequency of MAIT cells in liver compared to blood was observed in both normal and diseased states (Fig. 3A, B). The frequency of liver and blood MAIT cells in total CD3$^+$ T cells was decreased in chronic liver diseases (Fig. 3A, B). In liver as in blood, CD8$^+$ cells represented the major MAIT cell subset (Fig. 3C, D). However, in disease, the proportion of CD4$^+$ MAIT cells was significantly increased in both the blood and liver, which in liver, was compensated for by a significant reduction in the CD8$^+$ MAIT cell frequency (Fig. 3C, D). MAIT cells were unique among the T cell subsets that we examined in showing a reduced frequency with disease (Fig. 3E). We observed a negative correlation between total MAIT cells and total CD4$^+$ T cells in normal livers but found no sign of this correlation in disease. Conversely there was a trend towards a positive correlation of MAIT cells with CD8$^+$ T cells in normal livers. In non-autoimmune livers we noticed a positive correlation with CD161$^+$ T cells. No relationships were