Editorial

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Inflammation drives an altered phenotype of mucosal-associated invariant T cells in chronic hepatitis D virus infection

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Patients with chronic hepatitis B virus (HBV) infection are at risk of superinfection with the Hepatitis D virus (HDV), a small circular RNA virus that uses the HBV surface antigen (HBsAg) to envelope its genome and infect hepatocytes. Superinfection with HDV increases the severity of viral hepatitis, accelerates the progression to cirrhosis and increases the risk of developing hepatocellular carcinoma.[1] This process is thought to be immune-mediated, but the factors that drive disease pathogenesis are not well understood.

Tools to study the role of $CD8^+$ T cells in chronic HDV infection only recently became available when a comprehensive set of $CD8^+$ T cell epitopes was identified in 3 independent publications.[2–4] These studies also revealed the presence of HDV mutations in both individual patients and at the population level.[3,4] HDV mutations were associated with a memory-like phenotype of the corresponding $CD8^+$ T cells, consistent with the notion that stimulation via their HDV-specific T cell receptor (TCR) had ceased.[3] These findings imply that alternative mechanisms of immune cell activation may contribute to liver disease pathogenesis. Indeed, HDV but not HBV results in vigorous innate immune responses such as interferon (IFN)-β and IFN-λ-mediated induction of interferon-stimulated genes.[5,6] Innate immune cells with constitutively high expression of receptors for IFN-α/β and/or other inflammatory cytokines such as IL-12 and IL-18 constitute a large part of the intrahepatic infiltrate, even in the healthy liver. Of particular interest are mucosal-associated invariant T (MAIT) cells which comprise up to 40% of the intrahepatic immune cell population, even in the healthy liver. MAIT cells are innate-like T cells, typically identified by their expression of the C-type lectin CD161 and a semi-invariant TCR with conserved Vα7.2 chain. They are stimulated in a TCR-dependent manner by bacterial metabolites that are presented by the non-classical, monomorphic major histocompatibility complex (MHC) class I-related molecule MR1 and in a TCR-independent manner by inflammatory cytokines, especially IL-12 and IL-18.[7] Activation results in a rapid, innate-like response with secretion of antiviral and proinflammatory cytokines (IFN-γ, TNF-α and IL-17), and cytotoxic effector functions.[8] Owing to their innate-like response to cytokines MAIT cells display an activated phenotype in many inflammatory diseases, including HBV, HCV and HIV infections, alcohol-related liver disease, non-alcoholic steatohepatitis, primary sclerosing cholangitis and primary biliary cirrhosis [reviewed in [9]].

In this issue of the *Journal of Hepatology,* Dias *et al.* extend these findings to HDV infection, and show a significantly higher frequency of activated ($CD38^+$, $HLA\text{-}DR^+$ and $PD\text{-}1^+$) MAIT cells in the blood of HDV/HBV-coinfected patients than of HBV-monoinfected patients.[10] Unsupervised high dimensional analysis of flow cytometry data through stochastic neighbor embedding analysis revealed a compound phenotype of $CD38^{hi}PD\text{-}1^{hi}CD28^{lo}CD127^{lo}$ MAIT cells with low expression of the master transcription factors promyelocytic leukemia zinc finger protein (PLZF), eomesodermin (Eomes), and Helios in HDV/HBV-coinfected patients. As in the aforementioned inflammatory diseases, the frequency of MAIT cells was significantly reduced in the blood of HDV/HBV-infected patients compared to HBV-monoinfected patients and uninfected controls. Immunohistochemistry of 3 liver biopsies from HDV/HBV-coinfected patients and 3 controls without liver disease showed an accumulation of $CD3^+$ cells in the liver in HDV/HBV coinfection, but very few cells were identified as MAIT cells by co-expression of the IL-18Rα and the Vα7.2 TCR chain. Interestingly, HBV/HDV coinfected patients who received antiviral (nucleoside analogue) therapy against HBV had a slightly higher MAIT-cell frequency in the blood than untreated patients, and patients who had lost HBV e antigen (HBeAg) had a slightly higher MAIT-cell frequency than those who were HBeAg positive. This suggests that the reduced MAIT-cell frequency in the blood may be a reversible state dependent on the level of inflammation. This notion is consistent with a recent study,[11] which demonstrated that the reduced MAIT-cell frequency in the liver in chronic HCV infection improves within 4 weeks of therapy with direct-acting antivirals in parallel to normalization of alanine aminotransferase (ALT) activity.

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To assess MAIT-cell function, Dias *et al.* stimulated blood mononuclear cells of HDV/HBV-coinfected patients, HBV-monoinfected patients and uninfected controls with either fixed *E. coli* or with a combination of IL-12 and IL-18. *E. coli* was chosen as a representative for bacteria that synthesize vitamin B2 (riboflavin). Riboflavin biosynthesis generates the intermediate 5-amino-6-D-ribitylaminouracil (5-A-RU), which is presented by monocytes/macrophages on the MHC-related molecule MR1 to the semi-invariant TCR of MAIT cells.[12] In response to *E. coli* stimulation, MAIT cells from HDV/HBV-coinfected patients failed to upregulate CD69 and CD25 to the same extent as those of uninfected controls, produced less IFN-γ and degranulated less than those of HBV-monoinfected patients and uninfected controls. Whereas bulk MAIT-cell populations from all groups responded equally to stimulation with IL-12/IL-18, these responses were impaired for the PD-1$^+$ MAIT-cell subpopulation of HDV/HBV-coinfected patients. This links the MAIT-cell compound phenotype of chronic activation to dysfunction.

While this may indicate a differential response to TCR-mediated versus cytokine-mediated activation, it should be noted that the 24-hour incubation of blood mononuclear cells with *E. coli* also results in the release of autologous IL-12/IL-18.[11] Thus, the apparently differential response may reflect different cytokine concentrations in both stimulations. Indeed, IL-12/IL-18 stimulation of blood mononuclear cells from uninfected controls induced an abnormal MAIT-cell phenotype similar to the one observed in HDV/HBV-coinfected patients and resulted in MAIT-cell apoptosis. This is consistent with an innate-like transcriptional state of MAIT cells, where pre-formed mRNA for effector molecules allows rapid effector functions, but decreased baseline expression of ribosomal genes limits cell proliferation.[13] The notion of inflammatory cytokines driving MAIT-cell loss was consistent with increased blood levels of biomarkers of monocyte activation (soluble CD14 and soluble CD163), increased levels of the monocyte-derived cytokines (IL-18 and IL-12p40), and clinical markers of liver disease (increased ALT, aspartate aminotransferase, gamma glutamyltransferase levels, reduced prothrombin) that Dias *et al.* observed in HDV/HBV-coinfected patients.[10]

Whereas the authors clearly show significant alterations of the MAIT-cell compartment in chronic HDV/HBV coinfection, the immunological and clinical consequences require further investigation. This reflects the state of the larger MAIT-cell field, where the differential role of MAIT cells in immune protection and pathogenesis is currently one of the most intriguing areas of investigation. In mouse models, the reduced frequency of MAIT cells in the circulation is associated with recruitment of MAIT cells to infected tissues, where they contribute to the defense against pathogens.[14] For example, MAIT cells exert protective effects in bacterial (*F. tularensis*) and viral (influenza virus) infections of the lung.[15,16] Their rapid response is key to GM-CSF-mediated differentiation of inflammatory monocytes and to the recruitment and proliferation of IFN-γ-producing CD4$^+$ and CD8$^+$ T cells.[15] MAIT-cell responses are fine-tuned by the amount of riboflavin that is produced by microbiota. For example, Bacteroidetes and Proteobacteria phyla are known as high MAIT-cell stimulators, whereas members of the Firmicutes phylum are low/non-stimulators.[17] At the same time, there is active research in expanding the list of antigens that MAIT cells recognize, as it was shown that 5-A-RU, the intermediate of the vitamin B2-riboflavin biosynthesis pathway, does not fill the entire MR1 binding pocket [reviewed in [18]]. Known MR1 ligands now include intermediates of the vitamin B9-folate synthesis pathway, which inhibit MAIT-cell activation. Whether any of these ligands or the bacteria that synthesize them translocate from the gut to the liver in chronic hepatitis delta and contribute to the observed MAIT-cell phenotype needs to be determined in future studies.

Most importantly, the potential contribution of MAIT cells to the progression and pathogenesis of hepatitis delta needs to be assessed. While Dias *et al.* show that HDV itself does not directly affect MAIT cells, MAIT-cell function is known to be regulated by IL-7, a cytokine that hepatocytes secrete under inflammatory conditions. MAIT cells promote mitogenic and proinflammatory functions of fibrogenic cells[19] and thereby contribute to tissue-remodeling.[14] Accordingly, MAIT-cell-enriched mice show increased liver fibrosis, whereas MAIT-cell-deficient mice are fibrosis-resistant.[19] Finally, MAIT-cell activation in the liver may also contribute to tumor growth: liver MAIT cells produce IL-17, which promotes the differentiation of macrophages into the inflammatory M2 subtype, and – in a VEGF-mediated manner – stimulate angiogenesis [reviewed in [20]].

In summary, the study by Dias *et al.* is the first to analyze the phenotype and function of MAIT cells in chronic HDV/HBV coinfection. The cell subset analysis in the blood is well-performed and provides insights into the activation and dysfunction of MAIT cells in this disease. The authors clearly show that coinfection with HDV/HBV aggravates an inflammation-driven effect on MAIT-cell activation, frequency and function. An extension of this analysis to intrahepatic responses, particularly of patients with differential disease stages, is important to identify the mechanisms that contribute to the accelerated progression of liver disease in the presence of HDV.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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

Supplementary data

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Author names in bold designate co-first authorship

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