Expression of the immune checkpoints CD96 and CD226 on ascitic immune cells in patients with decompensated liver disease denotes a more immunosuppressive phenotype

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Background

Decompensated cirrhosis is associated with a marked susceptibility to infection with spontaneous bacterial peritonitis being one of the most common infections. Non-cirrhotic ascites rarely becomes infected so the degree and mechanism of immune-paresis in the ascitic immune environment is of particular interest.

Immune checkpoints constitute a complex array of regulatory receptors and ligands expressed on the surface of immune cells. They serve as pivotal regulators of the host immunity and so we hypothesise they may be involved in immune dysfunction in the ascitic immune environment.

TIGIT and CD96 are inhibitory receptors expressed on T cells and NK cells that are thought to exert immunosuppressive effects by outcompeting CD226, a co-stimulatory receptor also expressed on T cells and NK cells, for binding of its ligand CD155 on antigen presenting cells.

Methods

Patients with decompensated cirrhosis were recruited from the inpatient ward and healthy volunteers recruited as controls. Blood samples were collected for isolation of peripheral blood mononuclear cells (PBMC), and ascites mononuclear cells were obtained by centrifugation of ascitic fluid. Cell surface expression of the immune checkpoints CD96, TIGIT, and CD226 on T cells and NK cells, and their ligand CD155 on monocytes/macrophages was determined with flow cytometry analysis.

Healthy control PBMCs were cultured with TLR ligands and expression of CD155 on monocytes determined with flow cytometry analysis.

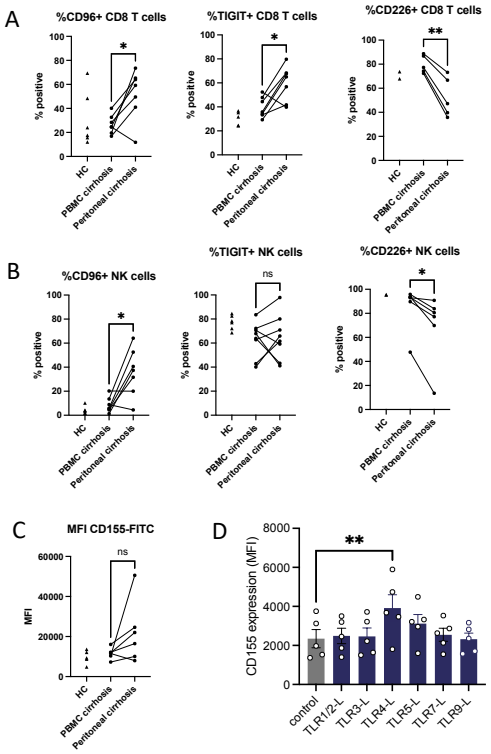
Results

A significantly higher percentage of ascitic CD8+ T cells and NK cells were CD96+ compared to paired peripheral cells from the same patient, whereas a significantly lower percentage were CD226+. There was a trend of higher CD155 expression on ascitic macrophages compared to peripheral monocytes. Healthy control monocyte expression of CD155 was significantly increased by TLR4 stimulation with the bacterial component lipopolysaccharide.

Expression of these immune checkpoints on ascitic immune cells did not significantly change with severity of liver disease from stable decompensation, acute decompensation and acute-on-chronic liver failure.

Discussion

The CD96, CD226 and CD155 expression on ascitic immune cells in decompensated cirrhosis is of a more immunosuppressive phenotype compared to corresponding peripheral immune cells. This may in part be explained by chronic background exposure to translocated bacterial products in the ascites and may pre-dispose to SBP.



(A) CD8+ T cells and (B) NK cells from healthy control PBMCs (n = 6) and decompensated cirrhosis PBMCs and ascites (n = 8). Percentage positive for CD96, TIGIT and CD226 as assessed by flow cytometry.
(C) Peripheral monocytes and ascitic macrophages CD155 expression (MFI).
(D) Healthy control monocytes (n = 5) cultured with TLR ligands and assessed for CD155 expression (MFI).
*Paired T test, **P < 0.01, *P <0.05*