Extracellular vesicles are involved in modulation of the immune system in minimal hepatic encephalopathy

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Background and Aims: Minimal hepatic encephalopathy (MHE) is associated with changes in the immune system including an increased pro-inflammatory environment and altered differentiation of CD4$^+$ T lymphocytes. The mechanisms remain unknown. Changes in extracellular vesicle (EVs) cargo including proteins and miRNAs could play a main role as mediators of immune system changes associated with MHE. The aim was to assess whether plasma EVs from MHE patients play a role in inducing the pro-inflammatory environment and altered differentiation of CD4$^+$ T lymphocyte subtypes in MHE patients

Method: We characterized the miRNA and protein cargo of plasma EV from 50 cirrhotic patients (27 without and 23 with MHE) and 24 controls. CD4$^+$ T cells from the controls were cultured with plasma EVs from the three groups of study, and the cytokine release were analyzed by ELISA. Differentiation to CD4$^+$ T-cell subtypes was assessed by quantifying the expression of specific transcription factors by qPCR.

Results: Plasma EVs from MHE patients had altered miRNA and protein contents, and were enriched in inflammatory factors compared to the controls and patients without MHE like MHC II, CD74, TNF-α and ADAM17 (Figure 1C and 1D). Plasma from patients with MHE was enriched in EVs from neurons (L1CAM), Th lymphocytes (CD4), Tc (CD8), and B lymphocytes (CD19) compared to those from the other studied groups (Figure 1A and 1B). EVs from MHE patients modulated the expression of pro-inflammatory IL-17, IL-21, and TNF-α and anti-inflammatory TGF-β in cultured CD4$^+$ T lymphocytes (Figure 2A and 2B), and increased the proportion of Th follicular and Treg cells and the activation of Th17 cells (Figure 2C).

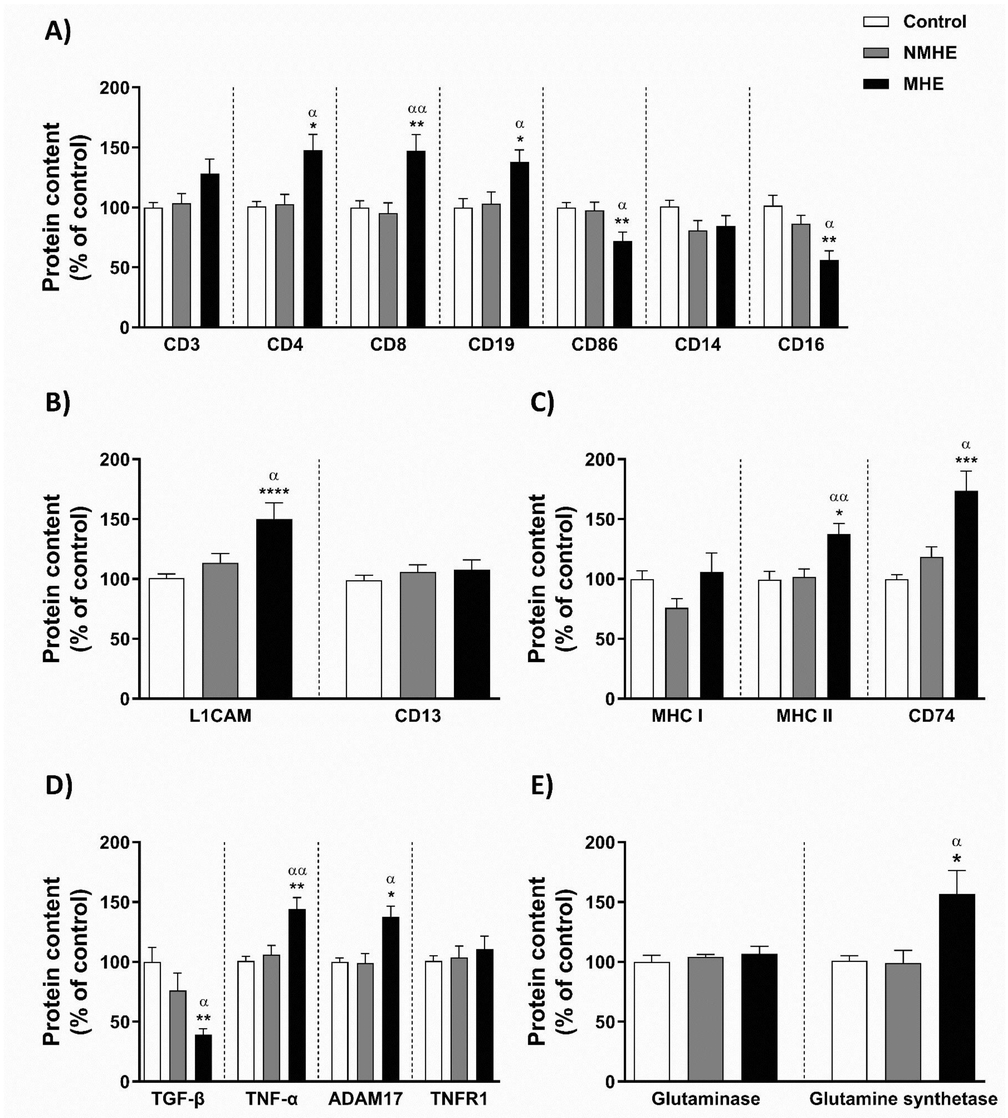


Figure 1. EVs protein cargo. Values are expressed as the percentage of the control and are the mean ± SEM. Values significantly different from the control are indicated by an asterisk (*) and from NMHE patients by α (*/α: $p < 0.05$; **/αα: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$).

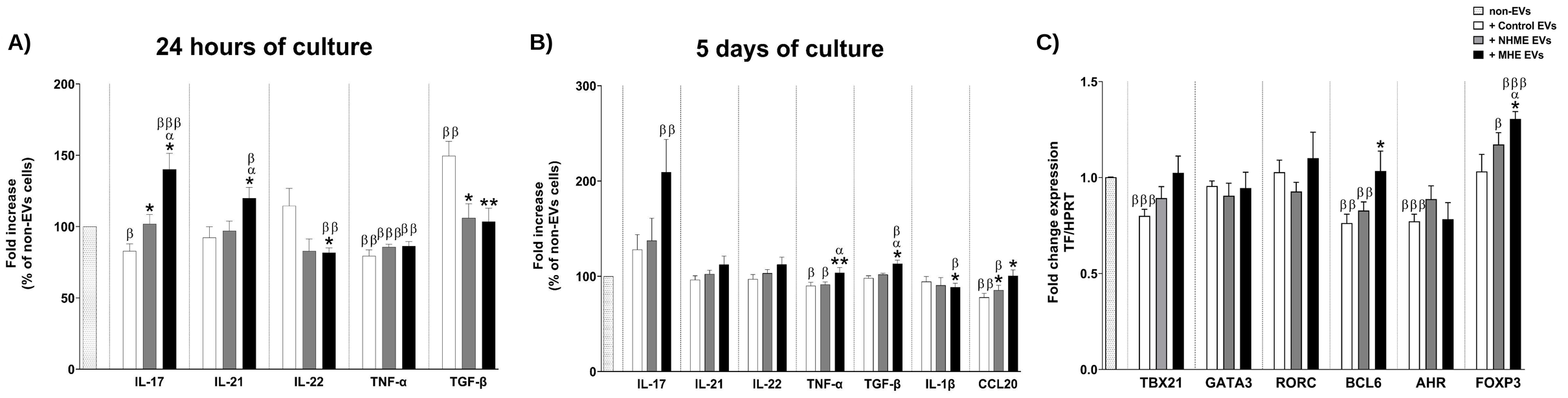
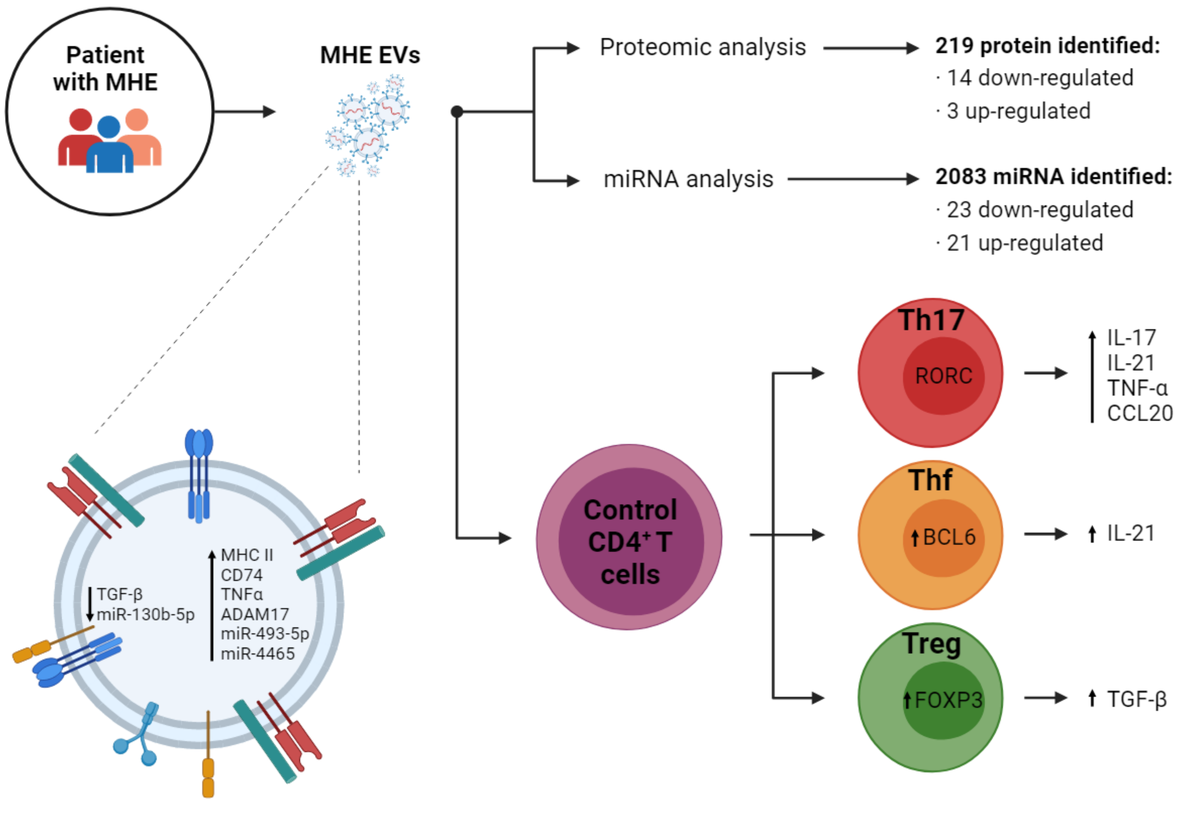


Figure 2. Cytokine release by CD4$^+$ T cells after **A)** 24 h and **B)** 5 days of culture and **C)** transcription factor expression in CD4$^+$ T cells after 5 days of treatment with human plasma EVs. A condition without EVs treatment was also included as a reference (non-EVs). Values are given as the fold increase of the cytokine and transcription factor expression levels over the non-EV condition, which was considered as 1. Values are the mean ± SEM. Eight independent experiments were conducted for all conditions. Values significantly different from the control EVs condition are indicated by asterisks (*), from the NMHE EV condition by α, and from the non-EVs condition by β (*/α/β: $p < 0.05$; **/ββ: $p < 0.01$; βββ: $p < 0.001$).



Conclusions: We have identified changes in protein content and miRNAs in EVs from patients with MHE compared to isolated EVs from control subjects and patients without MHE. These changes may be responsible for the effects observed in T CD4$^+$ lymphocyte cultures. The main changes observed in T CD4$^+$ lymphocyte cultures in the presence of EVs from MHE patients are increases in the cytokines IL-17, IL-21, TNF-α, CCL20 and TGF-β, and in transcription factors BCL6 and FOXP3, associated to follicular and regulatory T-lymphocytes, respectively, compared to cultures in the presence of EVs from controls or patients without MHE.

These changes are some of those seen in patients with MHE, suggesting that plasma EVs could play an important role in the induction of immune changes observed in MHE.

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