HIV/HCV Coinfection: Extracellular Vesicles Modulate NK Functionality in Relation to Liver Fibrosis

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BACKGROUND

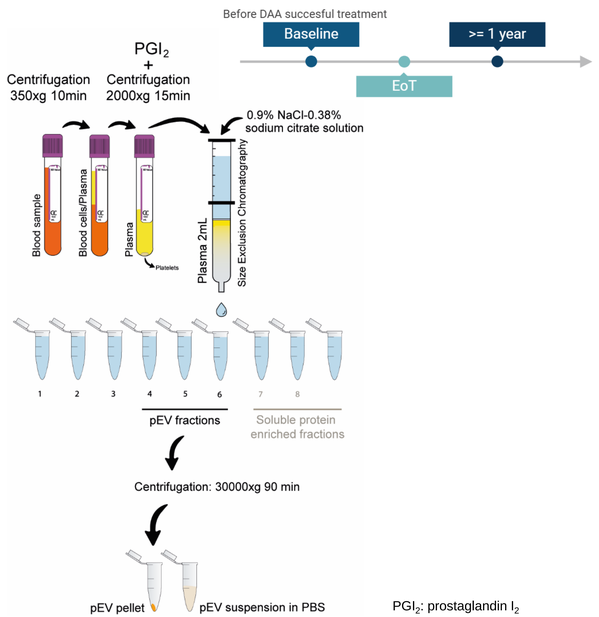
Plasma extracellular vesicles (pEV) play a role modulating the immune response. In **HIV/HCV coinfection**, **NK cells** are crucial in eliminating activated hepatocellular stellate cells, responsible for progression to **liver fibrosis (LF)**. **LF** can be staged in 5 different levels in the METAVIR scale, from F0 = no fibrosis, to F4 = cirrhosis.

We previously showed that in individuals with **advanced LF** (F4), **NK cells** presented an **exhausted** phenotype and **impaired functionality** even after elimination of HCV by direct-acting antivirals (DAA)[1].

Here, we aimed to characterize the effect of pEV from individuals with HIV/HCV and mild (F0/F1) or advanced LF (F4) on **NK cell functionality**.

METHODS

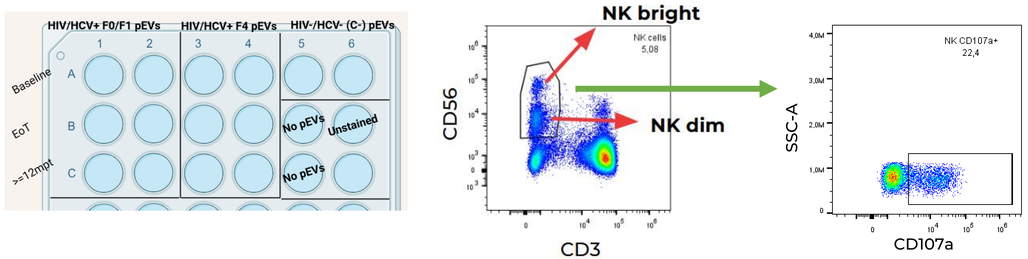
pEV were purified by size exclusion chromatography from 2 mL of plasma from F0/F1 (n=5), F4 (n=5) individuals at baseline (before DAA), EoT (end of treatment) and >= 1 year after successful treatment and from healthy controls (n=4), pEV were resuspended in PBS.



Plasma extracellular vesicles purification protocol. Figure adapted from Adamczyk & Leicaj et al, 2023[2].

Plasma extracellular vesicles (pEV) from individuals with *advanced liver fibrosis* (LF) exert a **higher suppressive effect on NK functionality** than pEV from individuals with *mild fibrosis* and healthy controls. This could be associated with the *little or no improvement in LF* observed in individuals with advanced liver fibrosis after HCV elimination by direct-acting antivirals.

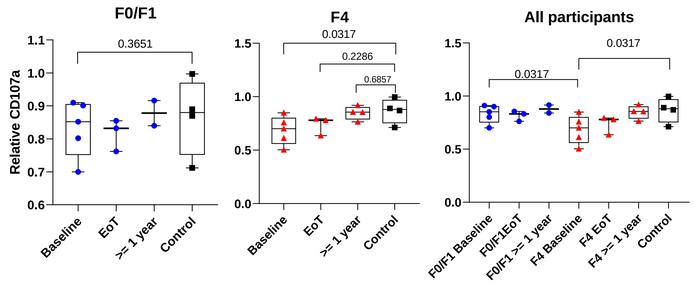
Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors and were plated in a 96 well plate (1x10^6 PBMC/well). PBMC were incubated for 1 hour with 30 uL of pEV suspension from each condition. After pEV incubation, PBMC were cocultured with K562 at a 10:1 ratio (1:1 NK:K562) for 2 hours. NK cell degranulation was evaluated by FACS analysis of CD107a, CD3, CD56 expression and a cell viability marker. We report NK relative degranulation as the ratio between NK CD107a+ in each condition and NK CD107a+ in absence of pEV. Data was analyzed using nonparametric statistics.



Coculture model used and cytometry analysis. PBMC from a healthy donor and K562 were added in all wells.

RESULTS

We found that pEV from all participants, including healthy donors, consistently reduced NK CD107a expression, compared to NK cells in the absence of pEV. However, pEV from individuals with advanced liver fibrosis exhibited a higher suppressive effect on NK cell functionality than those from healthy donors (0.70 vs 0.88; p=0.03) and individuals with mild fibrosis at baseline (0.70 vs 0.85; p=0.03). The inhibitory effect of pEV on NK cell cytotoxicity tends to be lower at one year or more after EoT than baseline, for both groups.



CONCLUSIONS

We show evidence that **pEV** from individuals with **advanced liver fibrosis** could play a role in the overall **exhausted phenotype** observed in **NK cells** from this group. This context could be one of the factors associated with **little or no improvement of LF** in individuals with advanced LF after **eliminating HCV by DAA treatment**. Further **characterization** of the composition of **pEV** could reveal **therapeutic targets** to reverse **NK cell dysfunction** and mitigate fibrosis in individuals with advanced liver fibrosis.

REFERENCES

1. Osegueda, A., et al. (2023). Markers of natural killer cell exhaustion in HIV/HCV coinfection and their dynamics after HCV clearance mediated by direct-acting antivirals. *Open Forum Infectious Diseases, 10*(1), ofad591. https://doi.org/10.1093/ofid/ofad591.

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