“Alcohol stimulates macrophage activation through caspase dependent, hepatocyte derived release of CD40L containing extracellular vesicles”

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on Behalf of TREAT Consortium

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Study Aims

To identify the mechanism by which ethanol metabolism by hepatocytes contribute to inflammation.

Abstract

Background/Aim. The mechanisms by which alcohol metabolism in hepatocyte activates macrophages in alcoholic liver disease (ALD) are unclear. Our prior *in vitro* studies with hepatocytes have shown Cyp2E1 dependent metabolism of ethanol stimulates release of macrophage activating nano- sized extracellular vesicles (EV) in a caspase 3 dependent manner. Here we sought to 1) identify hepatocyte derived EV content that may mediate macrophage activation and 2) extend this work into *in vivo* models.

Methods/Results. An unbiased microarray-based approach demonstrated high abundance of CD40 ligand (CD40L) in EV of ethanol treated Cyp2E1 overexpressing HepG2 hepatocytes (HepG2^{Cyp2E1}). Additionally, EV-induced macrophage activation was significantly abolished by more than 50% (TNF-α, IL-1β and IL-6 levels) upon antibody based CD40L neutralization in our *in vitro* assays with THP-1 cell line and primary macrophages. *In vivo* studies using chronic and binge ethanol feeding showed that administration of the pancaspase inhibitor IDN-7314, or mice with genetic deletion of CD40$^{-/-}$ or the caspase-activating TRAIL receptor (TR$^{-/-}$) were protected from ethanol induced macrophage activation compared with ethanol fed WT mice (p<0.05; N=5-10). Importantly, hepatic steatosis as quantified by microscopy and triglycerides analysis (p<0.05) and serum markers of liver injury were reduced in IDN-7314 administered and TR$^{-/-}$ mice compared to ethanol fed controls (p<0.05). Furthermore, mice on ethanol feeding had elevated EV (>2 fold) in their serum compared with control diet mice as quantified by nanoparticle-tracking analysis (NTA). This increase in EV numbers in alcohol fed mice was significantly reduced in serum of TR$^{-/-}$ or caspase inhibitor fed mice (p<0.05). Finally, serum from patients with alcoholic hepatitis also showed increased levels of CD40L enriched EV (p<0.05; N=6).

Conclusion. In conclusion, hepatocytes release CD40L containing EV in a caspase dependent manner in response to alcohol exposure which promotes macrophage activation, contributing to inflammation in ALD.

Results

Table-1: Different analytes detected in EV derived from alcohol treated HepG2^{Cyp2E1} cells

Analyte	Pixel Intensity
CD40 ligand	20754.6
IFN-gamma	17507.2
IL-23	17008.4
IL1-Ra	10295.0
PAI-1	7658.0
MIF	3768.8
IL-16	1766.9
MIP-1alpha	1571.0
GROalpha	1492.5
C5/5a	1434.3
I-309	1217.6
GM-CSF	1215.2
G-CSF	1162.7
sICAM-1	1127.1
IL-17e	1120.3
TNF-alpha	1089.1

Analyte	Pixel Intensity
IL-13	967.2
RANTES	874.8
IL1-alpha	839.7
sTREM-1	805.0
MCP1	702.4
IL-2	659.1
IL-4	566.2
MIP-1beta	514.7
IL-27	503.6
IL-17	452.1
IL-12p70	397.2
IL-6	388.5
IL-1beta	315.4
IP-10	294.7
IL-5	244.1
SDF-1	192.6
IL-10	155.5
IL-32alpha	122.8

Fig-1:CD40L neutralization is associated with reduced pro-inflammatory cytokines induction.

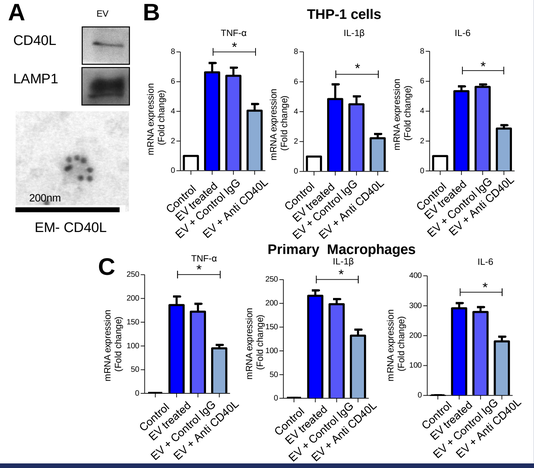


Fig- 2: *In vivo caspase inhibition suppresses EV release, reduces inflammatory cytokine production and macrophage activation.**

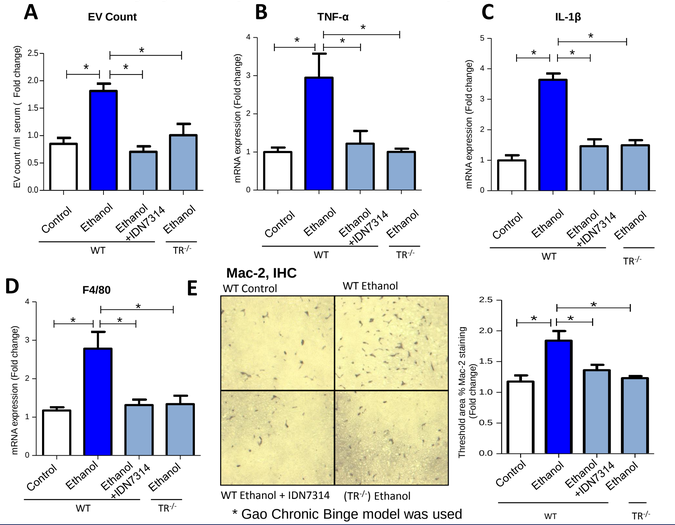
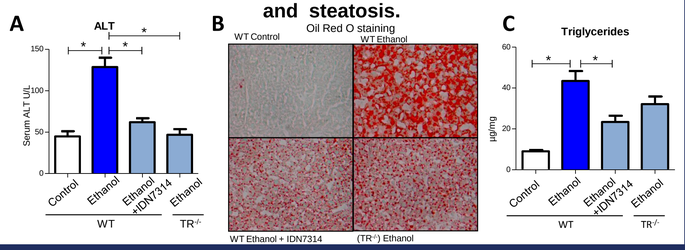
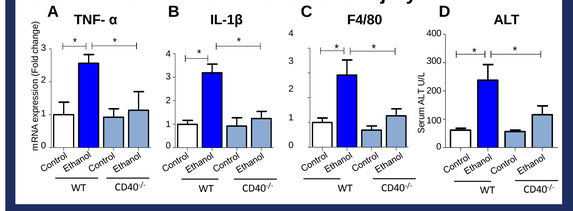


Fig- 3: Inhibition of EV release is associated with reduced Liver injury and steatosis.

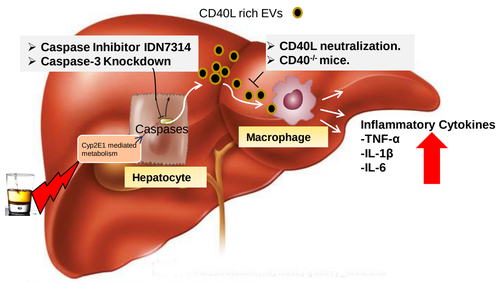


Results

Fig- 4: Mice lacking CD40L receptor are protected from ethanol induced inflammation and injury.



Summary



- Ethanol activates caspases in hepatocytes, thereby promoting extracellular vesicles (EV) release.
- EVs from ethanol-treated Hep^{Cyp2E1} are rich in CD40L and activates macrophages.
- *In vivo* blocking of caspase activation with pan-caspase inhibitor (IDN7314) or using mutants DR5$^{-/-}$ or CD40$^{-/-}$ mice was associated with reduced ethanol induced EV release and injury.