Examining The Effect of Inflammation on HDL-Cholesterol Uptake by the Liver

Aidan Hydomako, Dr. Mike Trites, Dr. Scott Widenmaier

University of Saskatchewan

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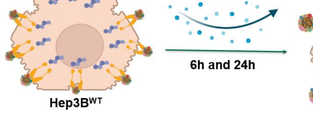
BACKGROUND

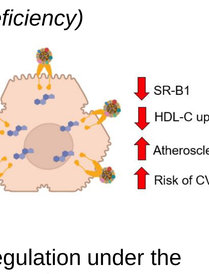
- Cholesterol is often stigmatized for its associations with cardiovascular diseases despite possessing crucial function in the body

Structure and Transport:

- Low-density lipoproteins (LDL) deliver cholesterol to peripheral cells
- High-density lipoproteins (HDL) return excess cholesterol to the liver in a process known as reverse cholesterol transport[7]

(Cholesterol Molecule)





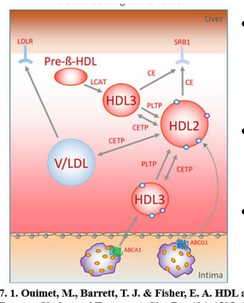
Consequences of Dysregulation:

- Cholesterol can accumulate in atherosclerotic plaques, increasing risk of heart attack and stroke[1]

Cytokines, Inflammation, and Atherosclerosis

- Inflammation is critical throughout atherogenesis[2]
- Pro-inflammatory cytokines like TNFα and their downstream signaling pathways are implicated in atherosclerosis-related inflammation[2]

SR-B1 Mediates HDL Uptake in the Liver



- Reverse cholesterol transport is promoted by HDL which moves excess cholesterol in the periphery back to the liver[7]
- Mice with disturbed SR-B1 function are predisposed to atherosclerosis[8]
- HDL and SR-B1 function therefore crucial in atheroprotection

7. 1. Ouimet, M., Barrett, T. J. & Fisher, E. A. HDL and Reverse Cholesterol Transport. Circ Res 124, 1505–1518 (2019).

OBJECTIVES

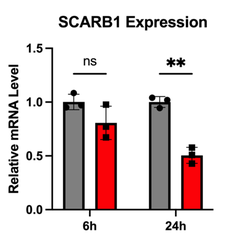
- In vitro, show that multiple treatment forms of inflammation significantly decrease SR-B1 expression in human hepatocytes

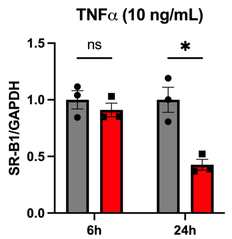
(Hypothetical Model of SR-B1 Deficiency)

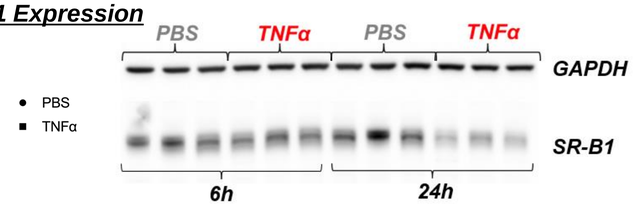
- Determine if the SR-B1 downregulation under the same inflammatory conditions translates to a decrease in the rate that HDL-cholesterol is taken up into cells
- Confirm that Dil-HDL uptake into the cells is mediated by SR-B1 using the inhibitor BLT-1
- Identify proteins critical for inflammation-induced signaling that reduces SR-B1 protein expression and potentially HDL uptake

RESULTS

1. 24h TNFα (10 ng/ml) Treatment Reduces SR-B1 Expression

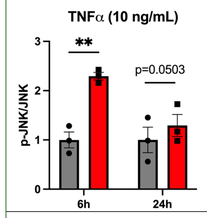


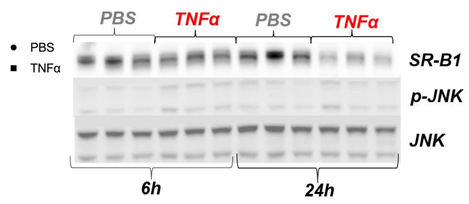




- mRNA levels falling after 6h of TNFα exposure ($p > 0.05$)
- 24h TNFα treatment downregulated expression of the SR-B1 gene ($p < 0.05$)
- Reduced mRNA levels after 24h translated to over a 50% reduction in SR-B1 protein levels

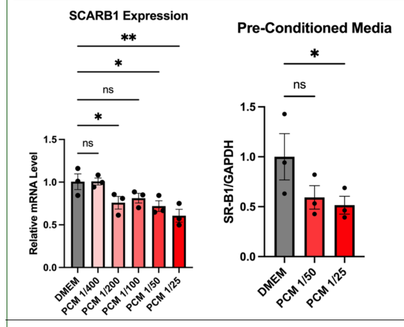
2. TNFα Treatment Activates JNK Signaling

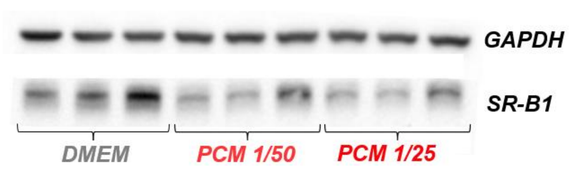




- Same proteins from TNFα experiment were used
- Over 2-fold elevation of p-JNK/JNK after 6h
- Results suggest decreased protein expression is delayed past 6h despite active pro-inflammatory signaling
- Near significant JNK activation at 24h ($p = 0.0503$)

3. Cytokine-rich Preconditioned RAW 264.7 Media Reduces SR-B1 Expression

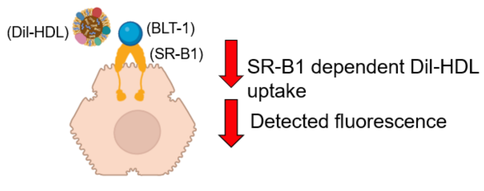


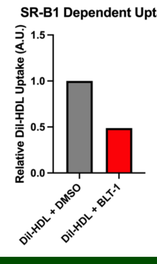


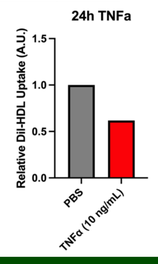
- 24h treatment only with ½ serial dilutions of stock macrophage media
- SR-B1 gene downregulated at doses of 1/200, 1/50, and 1/25 ($p < 0.05$)
- 24h treatment with macrophage media does downregulate SR-B1 gene expression
- SR-B1 protein expression significantly decreased at strongest dose (1/25)

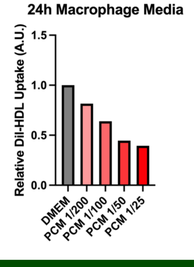
4. Inflammatory SR-B1 Deficient Conditions May Reduce Dil-HDL Uptake

(Model of SR-B1 Inhibition with BLT-1)









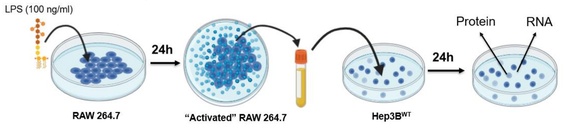
METHODS

Cell Culture → Gene and Protein Expression

- In vitro experimental model: Hep3B^{WT} (human hepatocytes) and RAW 264.7 (mouse macrophages)
- qPCR was used to quantify gene expression
- Western blot was used to determine changes in protein expression and quantified with ImageJ software
- Three independent experiments performed for results (1-3)

Macrophage Media Preparation:

- RAW 264.7 plated in serum free media and incubated with 100 ng/ml LPS for 24h at 37°C/5% CO_2



Statistics and Illustrations

- All analyses done in GraphPad Prism ($p<0.05$ considered significant)
- Uncited illustrations created with BioRender

Dil-HDL Fluorescent Uptake

- Biotek Synergy HT Plate Reader used to quantify fluorescence and determine relative uptake of Dil-HDL
- Fluorescence microscope with RFP filter used to obtain Dil-HDL images
- One independent experiment performed for results (4)

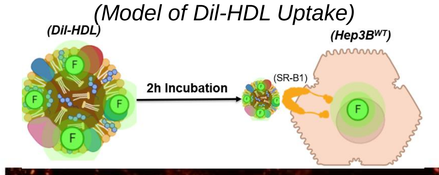
CONCLUSIONS

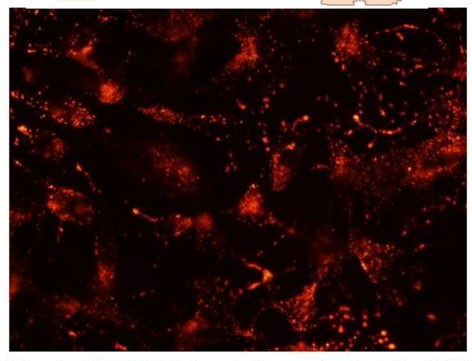
- In Hep3B^{WT}, TNFα and preconditioned macrophage media treatments significantly downregulate SR-B1 expression at the gene and protein level
- TNFα treatment activates JNK signaling in Hep3B^{WT} cells
- Reduced SR-B1 protein expression may translate to reduced SR-B1 dependent uptake of HDL-cholesterol based on preliminary Dil-HDL uptake data

NEXT STEPS

- Is SR-B1 downregulation in response to the 1/25 macrophage media primarily TNFα-dependent?
- With Dil-HDL imaging and uptake quantification, how do changes in SR-B1 expression modulate HDL uptake?

(Model of Dil-HDL Uptake)





(10ug/ml Dil-HDL 2h Incubation untreated Hep3B^{WT})

- Perform CRISPR-based genetic screen to identify protein mediators critical in inflammation-induced SR-B1 downregulation
- Based on screen results, generate knockout Hep3B^{WT} cell line(s)
- Deletion of identified critical gene(s) in knockout cell line(s) may protect against inflammation-induced SR-B1 deficiency in Hep3B^{WT} cells

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