Effects of Conjugated Linoleic Acid (CLA) on an *in vitro* model of Human Macrophage Differentiation as a Target of the CLA-induced Regression of Atherosclerosis

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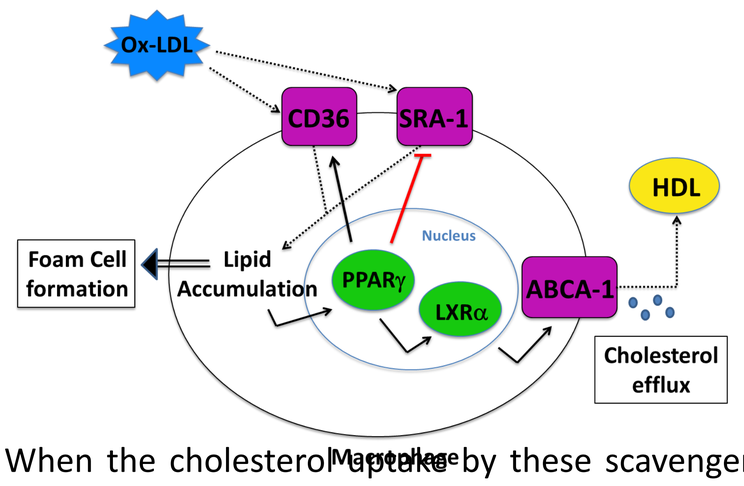
INTRODUCTION

Atherosclerosis is the underlying cause of heart disease and stroke characterized by the accumulation of lipids in large arteries[1].

Plaque lesion formation starts to occur with the accumulation of native or modified low density lipoproteins (LDL) by the macrophages which then turn into foam cells. Over time, plaque growth may block the blood flow in atheroprone regions of the vessel wall (particularly at branches and bifurcation sites).

On their surface, macrophages present several receptor families to traffic cholesterol. In particular, they use scavenger receptors (such as CD36 and SRA-1) for lipid uptake[2] and efflux proteins (such as ABC-A1 and ABC-G1) to remove cholesterol from the cell[3].

Macrophage colony stimulating factor (MCSF) causes monocytes to differentiate into a macrophage cell, of which CD14 is a pan-macrophage marker. The macrophage population is heterogeneous, and recently two main subtypes have been identified[4]. An M1 macrophage (characterized by high levels of ABC-A1) is pro-inflammatory, whereas an M2 macrophage (characterized by high levels of Mannose Receptor, MR) has anti-inflammatory properties.



When the cholesterol uptake by these scavenger receptors are at normal levels, the efflux proteins are able to pump the cholesterol back out the the cell and high density lipoproteins engulf the free cholesterol and are then able to transport it through the endothelium safely.

However, if there is an excess amount of cholesterol in the macrophage cell and the efflux proteins cannot remove them all, a foam cell is formed and a plaque lesion begins to develop.

CLA is a family of positional geometric isomers of linoleic acid (LA). They are produced as minor lipid fractions in ruminant animals. The predominant naturally occurring isomers are *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA (*c*-9,*t*-11 : *t*-10,*c*-12 = 80:20 ratio, which is the proportion used for the CLA blend in our experiments)[5].

Work in the Belton Laboratory has previously shown that CLA induces regression of pre-established atherosclerosis in ApoE$^{-/-}$ mice[6].

However, the exact mechanism(s) through which CLA alters macrophage functions has not been fully elucidated.

RESEARCH AIMS

- To determine the effects of CLA on modulation of gene expression of scavenger receptor, efflux protein and monocyte differentiation markers in macrophages.

STRATEGY

- Monocyte/Macrophage cells were treated with 10uM of *c*-9,*t*-11 CLA, *t*-10,*c*-12 CLA isomers and a CLA blend (80:20).
- Troglitazone (TROG 5uM) an agonist of PPARγ nuclear receptor was used to determine the mechanism of action through which CLA works. 10uM of Oleic acid (OA) and Linoleic acid (LA) were used as fatty acid controls.

RESULTS

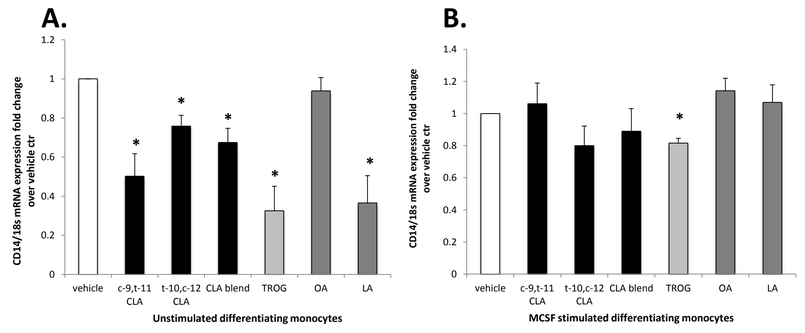


Figure 1. Effects of CLA on the CD14 pan-macrophage marker mRNA expression. These results show that CLA decreases CD14 expression in unstimulated monocytes **(A)**, but the CLA effect on CD14 is then lost in the MCSF stimulated cells **(B)**. In the absence of differentiating stimulus, CLA behaves similarly to TROG, possibly acting dependent to PPARγ activation.

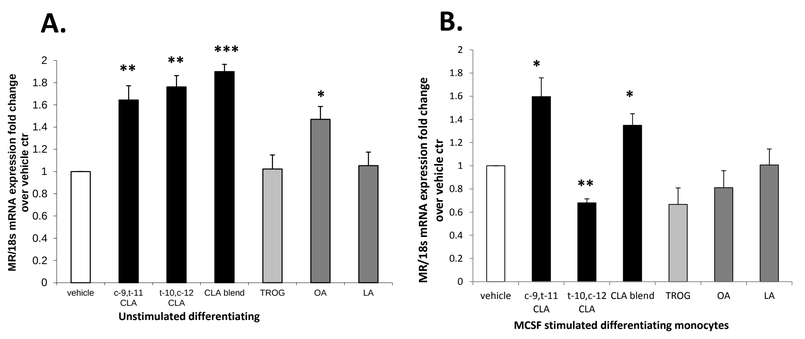


Figure 2. Effects of CLA on the M2 macrophage marker MR mRNA level. CLA upregulates MR gene expression independently of MCSF stimulation. In resting conditions **(A)** both isomers and their blend increase MR expression. The presence of MCSF **(B)** inhibits the *t*-10,*c*-12 increasing effect. CLA acts independently from PPARγ and the effect is specific to CLA, and not common to any fatty acid.

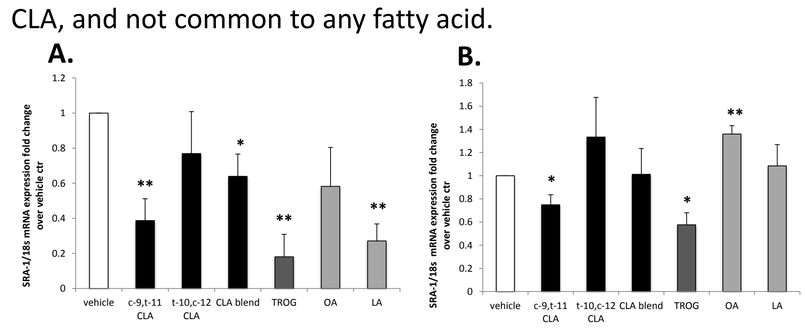


Figure 3. Effects of CLA on the SRA-1 scavenger receptor marker mRNA expression. RT-PCR results show that CLA downregulates the expression of SRA-1. In both cases, CLA behaves similarly to TROG, so possibly following a PPARγ-dependent mechanism. In the absence of MCSF **(A)**, LA has similar effects to CLA but presents an opposite effect when the macrophage is stimulated with MCSF **(B)**.

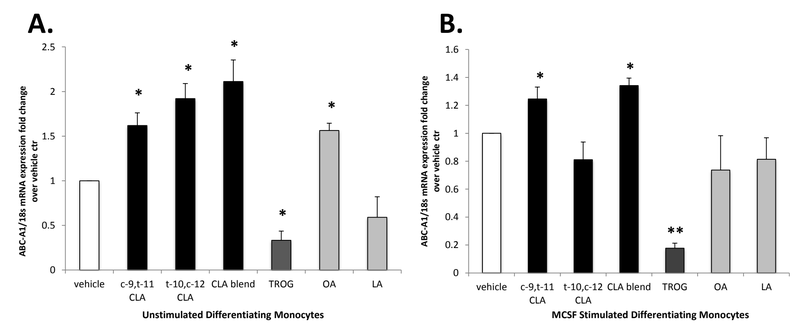


Figure 4. Effects of CLA on the ABC-A1 M1 macrophage and efflux protein marker mRNA expression. RT-PCR results show that both CLA isomers and their 80:20 blend increase the expression of ABC-A1 in unstimulated monocytes **(A)**, whilst, in MCSF-stimulated monocytes **(B)**, the *t*-10,*c*-12 CLA upregulating effect is prevented. In both conditions, CLA acts differently from TROG and from the fatty acid controls (except OA in unstimulated conditions).

METHODOLOGY

- Reverse transcription was run on human total RNA samples using Superscript III enzyme to obtain a target cDNA concentration of 500ng.
- Real Time PCR was performed on the cDNA samples. Different primers were used for each of the different target genes, either using TaqMan or SyberGreen Assays. These are fluorescent probes that can detect and quantify the target cDNA sequences.

DISCUSSION

- During early stages of differentiation (such as in the absence of MCSF stimulation) the main target of CLA is CD14. In particular, CLA downregulates this pan-macrophage marker, possibly following a PPARγ-dependent mechanism.
- In both stimulating conditions, CLA isomers upregulate MR expression by inducing an M2 macrophage phenotype. This effect is independent from both MCSF and PPARγ activation.
- In both resting and MCSF-stimulated cells, CLA inhibits mRNA expression of SRA-1, possibly following a PPARγ-dependent mechanism. In the presence of MCSF stimulus, the CLA effect is less prominent.
- In both stimulating conditions, CLA induces ABC-A1 expression, with different extent of the effect between resting and MCSF-activated conditions. These effects however do not depend on PPARγ activation.

CONCLUSION

- These results show that CLA induces an M2 anti-inflammatory macrophage phenotype, characterized by low expression of CD14 and high levels of MR, characteristic of a phagocytic activity.
- Lipid uptake is prevented by CLA inducing lower expression of the scavenger receptor SRA-1. Moreover, the removal of cholesterol from the macrophage cells is induced by CLA upregulating ABC-A1 efflux protein surface levels.

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