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### Malondialdehyde Acetaldehyde Adducts (MAA-Adducts) Direct Distinctive Pro-Inflammatory Responses in Endothelial and Macrophage Cell Lines

Patrick J. Opperman

Michael J. Duryee

Ted R. Mikuls

Geoffrey M. Thiele

Dahn L. Clemens

See next page for additional authors

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### Authors

Patrick J. Opperman, Michael J. Duryee, Ted R. Mikuls, Geoffrey M. Thiele, Dahn L. Clemens, and Daniel R. Anderson



## **ABSTRACT**

Chronic inflammation plays a critical role in the pathogenesis of atherosclerosis. At present, the mechanism(s) by which inflammation contributes to this disease is not entirely understood. Inflammation is known to induce oxidative stress, of which one consequence is lipid peroxidation. This process leads to the production of malondialdehyde (MDA), which can subsequently break down to form acetaldehyde (AA). These two aldehyde by-products can covalently interact with the ε -amino group of lysines within proteins and lipoproteins leading to the formation of highly immunogenic malondialdehyde-acetaldehyde adducts (MAA-adducts). The aim of this study was to determine the in-vitro cytokine response of endothelial cells and macrophages treated with MAA-modified human serum albumin (HSA-MAA) and low-density lipoprotein (LDL-MAA). In addition, cells isolated from mice with exposure to MAA and high fat diets were stained and imaged for uptake of the modified macromolecules of interest. We found that exposure of endothelial cells resulted in increased expression of IL-6, TNF- α, ICAM-1, VCAM-1, and MCP-1 in response to incubation with HSA-MAA; whereas, the same treatment of macrophages resulted in increased expression of IL-6, TNF- $\alpha$ , and IL-1 $\beta$ . LDL-MAA incubation resulted in increased TNF- $\alpha$  expression in macrophages, but MCP-1 was elevated in endothelial cells. Interestingly, the quantitative and qualitative uptake of triglycerides was increased in both endothelial and macrophage cells when exposed to LDL-MAA compared to LDL alone. The results of these studies demonstrate that different MAA-adducts elicit unique responses in different cell types. Additionally, the presence of MAA appears to modulate the cells leading to increased uptake of triglycerides and further progression of the inflammatory response.

# atherosclerosis.

- oxygen species.
- Oxidative stress can lead to lipid peroxidation. Lipid peroxidation results in the production of malondialdehyde (MDA) and acetaldehyde (AA).
- MDA and AA can covalently interact with the  $\varepsilon$ -amino group of lysines within proteins and lipoproteins forming highly stable and immunogenic malondialdehyde-acetaldehyde adducts (MAA-adducts).

- or LDL-MAA.

# Malondialdehyde Acetaldehyde Adducts (MAA-Adducts) Direct Distinctive Pro-Inflammatory Responses in Endothelial and Macrophage Cell Lines

Patrick J. Opperman, Michael J. Duryee, Ted R. Mikuls, Geoffrey M. Thiele, Dahn Clemens, and Daniel R. Anderson University of Nebraska Medical Center - College of Medicine - Divisions of Cardiology and Rheumatology

# BACKGROUND

Cardiovascular disease (CVD) is one of the leading causes of death in the world. It is known that chronic inflammation is a key mediator of the pathogenesis of

Common risk factors for cardiovascular disease – hypertension, hyperlipidemia, smoking, diabetes, and obesity – are associated with increased levels of reactive

MAA adducts have been identified in a ortic atheromas of patients with CVD and antibody titers to MAA-adducts are associated with disease severity in CVD. Given this knowledge, identifying the role of MAA-adducts in the pro-inflammatory cytokine response and role in development of the cholesterol plaque is key.

### PURPOSE

To determine the pro-inflammatory response of endothelial and macrophage cell lines to MAA-modified macromolecule incubation

### METHODS

In vitro cell culture of endothelial (CRL 2167) and macrophage (J774) cell lines was performed; and, these lines were incubated with HSA, HSA-MAA, LDL, and LDL-MAA.

The cells were processed for RNA isolation and then reverse transcribed into cDNA prior to RT-PCR analysis to determine mRNA expression of IL-6, TNF-  $\alpha$ , IL-1 $\beta$ , ICAM-1, VCAM-1, and MCP-1. Mice were treated with MAA adducted albumin or no injection and fed chow or high fat diets. Then, cells were extracted and stained to determine uptake of triglycerides in the presence of unmodified LDL

Students T-Test was used to determine differences between groups using GraphPad Prism 7.04 Software.

> This study confirms prior studies which demonstrate that oxidative stress leads to increased antibody levels (anti-MAA Ab) in response to elevated levels of lipid peroxidation (Data not shown). > Incubation of the endothelial cell line demonstrates a clearly elevated pro-inflammatory cytokine response to HSA-MAA, but not in response to LDL or LDL-MAA (Fig. 1, Panel A). This perpetuates the inflammatory response and is seen with concomitant increases in adhesion molecules and monocyte chemoattractant protein. These are key components in the pathway for development of a cholesterol plaque. > The macrophage cell line demonstrates an elevated pro-inflammatory cytokine response to HSA-MAA; however, there is also a significant increase in TNF in response to LDL-MAA incubation (Fig. 1, Panel B). > These findings are important as they help corroborate and classify the pro-inflammation. In tandem, this study shows that MAA modified LDL increases the uptake of triglycerides into both endothelial and macrophage cells (Fig. 2, A & B). > Animal studies demonstrate a clear increase in the anti-MAA adducts to increase this response. Clearly these MAA adducts are in part perpetuating the immune response, thereby increasing the susceptibility of inducing plaque formation in the vessels (Fig. 3, A & B).









<sup>©</sup> 4×10<sup>2</sup>-

≥ ຣ 3×10².

2×10<sup>2</sup>

## RESULTS

### Figure 2. Qualitative & Quantitative Assessment of LDL Uptake in Endothelial and Macrophage Cell lines. Figure A demonstrates endothelial cell uptake of triglycerides using Oil Red O stain in the images to the left and quantitative assessment in the graph on the right. Figure B demonstrates macrophage cell uptake of triglycerides using Oil Red O stain in the images to the left and quantitative assessment on the right.



# CONCLUSIONS

Figure 3. Serum Anti-MAA antibodies following high fat diet and **immunization with MAA protein adduct.** Figure A demonstrates the antibody concentration in animals with no injection. Figure B demonstrates the antibody concentration in animals following immunization with MAA-adduct. Note the scale between graphs and the dramatic increase in anti-MAA antibodies following immunization in the high fat animals compared to controls. N=5