5-12-2015

Programming Macrophage Inflammation Resolution: The Role of Omega-3 Polyunsaturated Fatty Acids

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Abstract
It was previously thought that resolution of inflammation was a passive process, but recent emerging research has identified that resolution is an active process and that dual acting lipid mediators derived from essential omega-3 polyunsaturated fatty acids (PUFAs) have both anti-inflammatory (reducing neutrophil access to the inflamed tissue) and pro-resolving (removal of apoptotic cells by macrophages in the inflamed site) actions. The objective of our study was to determine the role of omega-3 PUFAs in programming phenotypic changes in treated macrophages. The polarization of macrophages during inflammatory responses to functionally distinct phenotypes may play a role in both inflammation and resolution of inflammation following treatment with omega-3 PUFAs for chronic inflammatory diseases.

Keywords
fsc2015

Disciplines
Pharmacy and Pharmaceutical Sciences

Comments
Presented at LIPID MAPS (LIPIS Metabolites and Pathways Strategy) Annual Meeting 2015: Lipidomics Impact on Cancer, Metabolic, and Inflammatory Diseases in La Jolla, California, May 2015.

Programming Macrophage Inflammation Resolution: The role of omega-3 polyunsaturated fatty acids

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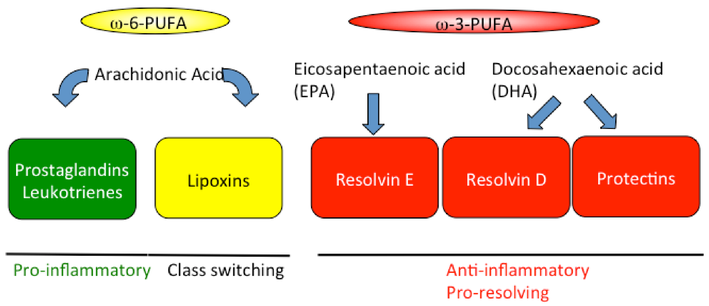
Introduction

The objective of our study was to determine the role of omega-3 polyunsaturated fatty acids in programming phenotypic changes in treated macrophages

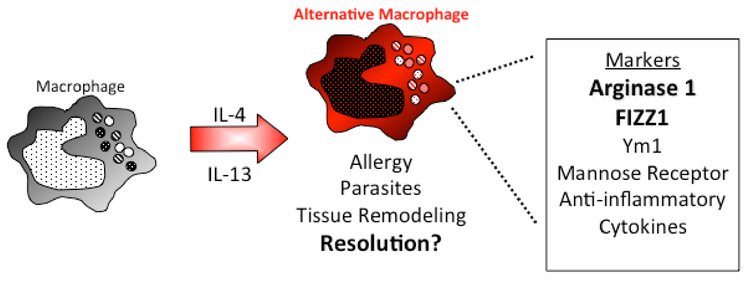


➢It was previously thought that resolution of inflammation was a passive process, but recent emerging research has identified that resolution is an active process and that dual acting lipid mediators derived from essential ω-3 PUFAs have both anti-inflammatory (reducing neutrophil access to the inflamed tissue) and pro-resolving (removal of apoptotic cells by macrophages in the inflamed site) actions.

➢Omega-3 PUFAs have anti-inflammatory and pro-resolving activity in pre-clinical disease models (e.g. peritonitis, colitis, asthma)

➢Chronic inflammation is a key factor in the pathogenesis of numerous diseases (e.g. COPD, RA, IBD)

The M2 Macrophage Phenotype

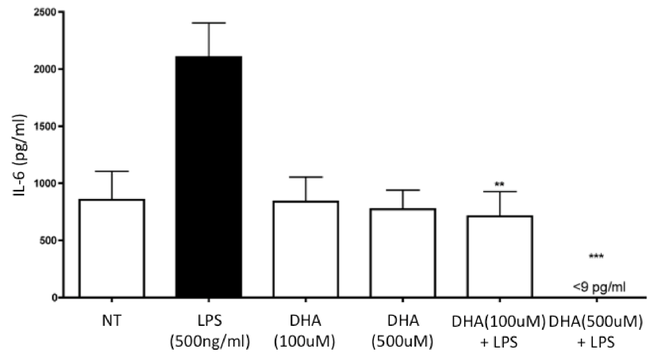


➢Role in allergic responses driven by Il-4 and IL-13

➢Involved in the development of TH2-dependent immune response to extracellular parasites

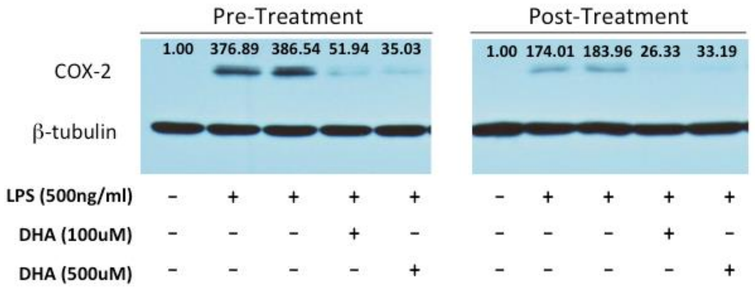
➢M2 macrophages are associated with an anti-inflammatory state

DHA treatment reduces IL-6 production in product in LPS-stimulated RAW264.7 macrophages



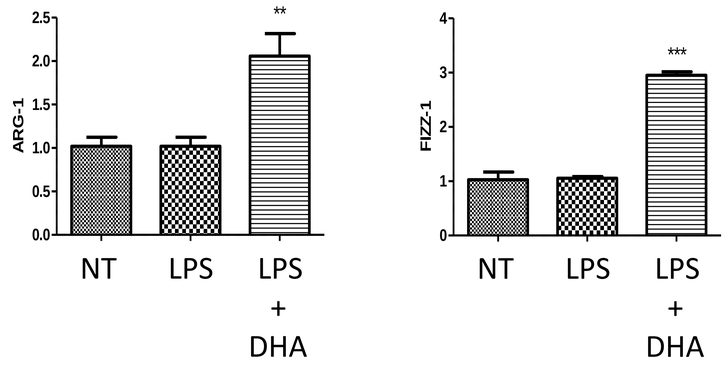
IL-6 production was measured in the supernatant fraction of treated cells by ELISA. RAW264.7 macrophages were seeded at a density of 200,000 cells/ml and pre-treated with the indicated concentrations (in µM) and combinations of ω-3 PUFAs ~18 hours prior to a 24 hour LPS (1 µg/ml) stimulation. Data is represented as mean ± SEM for *n*=3. ***p*<0.01 versus LPS-treated cultures by one-way ANOVA; ****p*<0.001 versus LPS-treated cultures by one-way ANOVA. (DHA (500uM) + LPS = not detected by lower sensitivity of assay.)

Exposure to DHA following inflammatory insult attenuates COX-2 expression on LPS-stimulated RAW264.7 macrophages



Western blot: RAW264.7 macrophages were seeded at a density of 200,000 cells/ml and pre-treated with DHA ~18 hours prior to a 24 hour LPS (500ng/ml) stimulation or treated with DHA 30 minutes after LPS stimulation. Non-treated cultures were treated with the same final concentration of ethanol as vehicle. Cell lysates were collected and subjected to western blot analysis using an anti-COX-2, or anti-beta-tubulin antibody. The antibody-specific bands were quantified by densitometry and were normalized to beta-tubulin (numerical values above COX-2 band.) Blot shown is a representative image from 3 independent experiments.

DHA drives polarization of alternatively activated (M2) macrophages



Quantitative RT-PCR. Total RNA was extracted from non-treated, LPS treated (500 ng/ml), and DHA pre-treated (500 uM) RAW264.7 macrophages to measure mRNA levels of Arg-1 and FIZZ-1. Data is represented as mean ± SEM for n=4. **p<0.01 versus LPS-treated cultures by one-way ANOVA; ***p<0.001 versus LPS-treated cultures by one-way ANOVA.

Conclusions

➢The action of ω-3 PUFAs drives the decrease in the proinflammatory mediator COX-2 and IL-6

➢M2 markers, Arg-1 and FIZZ1, are up-regulated in DHA treated macrophages

➢The polarization of macrophages during inflammatory responses to functionally distinct phenotypes may play a role in both inflammation and resolution of inflammation following treatment with ω-3 PUFAs.