Selective Utilization of the Phospholipid and Triacylglycerol Pools of Arachidonic Acid in Murine Macrophages

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Innate immune cells, including monocytes and macrophages, possess substantial amounts of arachidonic acid (AA), which can be mobilized during activation to generate a wide range of bioactive oxygenated compounds. In inflammatory environments, these cells typically accumulate significant AA not only within membrane phospholipids but also in neutral lipids such as triacylglycerols. This prompted investigation into the metabolic pathways of these two distinct AA reservoirs in macrophages. Using various radiolabeling approaches to differentiate between the phospholipid and triacylglycerol compartments, we demonstrate that upon acute stimulation with yeast-derived zymosan, the phospholipid-bound AA serves as the primary—if not sole—source of free AA. Conversely, AA stored in triacylglycerols appears to be utilized during the resolution phase of the zymosan response, functioning to replenish the depleted phospholipid stores. Therefore, phospholipids and triacylglycerols fulfill distinct roles in AA turnover and regulation during macrophage activation.

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Slide 1. Title.

Slide 2. Role of Phospholipase A_2 in Arachidonic Acid Release.

Slide 3. Lipid Droplets: Yesterday and Today.

Slide 4. Lipid Droplets as Sources of AA for Lipid Mediator Production.

Slide 5. Arachidonic Acid Distribution in Murine Macrophages. (A) AA incorporation into the lipids of mouse peritoneal macrophages. The cells were either untreated (open bars) or treated with 20 μM AA (orange bars). Afterward, the various lipid classes were isolated and their AA content was measured by GC-MS. (B) The cells, prelabeled with [3H]AA, were either untreated (open symbols) or treated with 0.5 mg/ml zymosan (black symbols) for the times indicated. Afterward, the extracellular media were removed and analyzed for radioactivity content

Slide 6. Contribution of PL and TAG Pools to Overall AA Release. The [3H]AA-labeled cells were either untreated (Ctrl) or stimulated with 0.5 mg/ml zymosan for 2 h in the absence (Zym) or presence of 1 μM pyrrophenone (Zym + Pyrr), or 20 μM atglistatin (Zym + AGS), or 10 μM bromoenol lactone (Zym + BEL). Afterward, the [^{3}H]AA released to the supernatants (A) or remaining in phospholipids (B) or TAG (C) was quantified.

Slide 7. Source of AA release in activated macrophages. The cells, labeled with [^{3}H]AA and [^{14}C]AA were treated with 0.5 mg/ml zymosan for 1 h. Afterward, the ^{3}H/^{14}C ratio of extracellularly liberated free AA was calculated and compared to cellular PL and TAG ratios.

Slide 8. Transfer of AA from Triacylglycerol to Phospholipids. Cells, prelabeled with 20 µM [^{3}H]AA for 2 h were either untreated (A) or stimulated with 0.5 mg/ml zymosan for 2 h (B). Afterward they were washed and transferred to fresh media, and the distribution of [^{3}H]AA content in phospholipids (maroon symbols) and TAG (open symbols) was determined at the times indicated.

Slide 9. The phospholipid pools likely constitute the major, if not the only, source for releasable AA under acute stimulation conditions.

Slide 10. (Transition slide).

Slide 11. AA-containing in TAG pools help replenish the phospholipid pools that have been exhausted after acute stimulation.

Slide 12. New Role: The AA pool in TAG contributes to regulating AA metabolism and dynamics. Biological specificity – Compartmentalization.

Slide 13. Acknowledgments

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Our work reveals distinct functional roles for arachidonic acid (AA) reservoirs within macrophages. The findings suggest that, during acute stimulation, phospholipid-bound AA serves as the principal—if not exclusive—source of mobilizable AA. In contrast, the AA stored in triacylglycerols (TAG) appears to follow a different metabolic trajectory: it contributes to the replenishment of phospholipid stores depleted during early activation phases. Consequently, the AA pool in neutral lipids may play a supportive role in modulating AA turnover and maintaining lipid homeostasis during innate immune responses. A comprehensive list of significant papers from our lab follows.

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