Lipid Droplet Formation Regulated by Group IVA Phospholipase A\(_2\)

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Exposure of human peripheral blood monocytes to free arachidonic acid (AA) results in the rapid induction of lipid droplet (LD) formation by these cells. LDs are formed by two different routes, namely (i) the direct entry of AA into triacylglycerol and (ii) activation of intracellular signaling leading to increased neutral lipid formation utilizing fatty acids coming from the de novo biosynthetic route. The latter predominates, accounting for 60-70% of total LD formation, and can be completely inhibited by selective inhibition of the group IVA cytosolic phospholipase A\(_2\)\(\alpha\), pointing out this enzyme as a key regulator of AA-induced signaling. LD formation in AA-treated monocytes can also be blocked by the combined inhibition of the mitogen-activated protein kinase family member p38 and JNK, which correlates with inhibition of cPLA\(_2\)\(\alpha\) activation by phosphorylation. Since cPLA\(_2\)\(\alpha\) has no effect on neutral lipid formation there must be a step in the mechanism of LD biogenesis that is critically dependent on the enzyme, e.g. generation of a positive curvature at the organelle baseline.

Slide 1 – AA-induced LD formation in human monocytes. Monocytes, treated without (left column) or with (right column) 3 µM triacsin C for 30 min, were exposed to AA, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), or \(\gamma\)-linolenic acid (\(\gamma\)18:3) for 2 h as indicated. All fatty acids were added at a concentration of 10 µM. After fixation and permeabilization, cells were stained with BODIPY493/503 (2 µg/ml) to visualize LD (green; right panels) and DAPI (1 µg/ml) to mark the nuclei (blue; central panels). Left panels show the Nomarski images.

Slide 2 – Effect of triacsin C on the incorporation of fatty acids into TAG in human monocytes. A: Effect of triacsin C on the distribution of fatty acids in TAG after treatment of the monocytes with 10 µM AA for 2 h. The analysis of TAG fatty acids was carried out by GC-MS after converting the fatty acid glyceryl esters into fatty acidmethyl esters. B: Total cellular TAG values and the result of adding the masses of all fatty acids under each condition and dividing by 3. Data are given as means ± SE of three independent experiments. *Significantly different (p < 0.05) from their respective controls.

Slide 3 – Effect of various inhibitors on AA-induced LD formation. Effect of various inhibitors on AA-induced LD formation. Monocytes, preincubated with 3 µM triacsin C for 30 min, were untreated (left column) or treated with 10 µM AA for 2 h (right column) in the presence of the indicated inhibitor at the following concentrations: 1 µM pyrrophenone, 10 µM SB203580, and 10 µM SP600125. After fixation and permeabilization, cells were stained with BODIPY 493/503 (2 µg/ml) to visualize LD and with DAPI (1 µg/ml) to mark the nuclei.

Slide 4 – Stimulation of mitogen-activated protein kinases and cPLA\(_2\)\(\alpha\) by AA in human monocytes. A: Monocytes were treated without (Control) or with 10 µM AA for the indicated times and analyzed for expression of phosphorylated p38 and JNK by immunoblot. B: Analysis of the kinases implicated in cPLA\(_2\)\(\alpha\) phosphorylation. Monocytes were treated with 10 µM AA for 2 h as indicated. Some of the samples were
preincubated with the following specific kinase inhibitors as indicated: 10 µM PD98059, 10 µM SB203580, 10 µM SP600125, or 10 µM SB203580, plus 10 µM SP600125. All incubations proceeded in the presence of 3 µM triacsin C. Phosphorylation of cPLA\(_2\alpha\) at Ser 505 was analyzed by immunoblot. The Western blots for phosphorylated p38, JNK, and cPLA\(_2\alpha\) were quantified from three different experiments (means ± SE).

Slide 5 – LD synthesis from the cytosolic face of the smooth endoplasmic reticulum membranes. LD synthesis from the cytosolic face of the smooth endoplasmic reticulum membranes. The essential role of cPLA\(_2\alpha\) in inducing a positive membrane curvature is indicated in step 3. Lysophospholipids are highlighted in pink, and phosphatidic acid in green. All other phospholipids are shown in blue. For further details see text. DGAT, diacylglycerol:acyl-CoA acyl transferase; ACAT, acyl-CoA:cholesterol acyl transferase; ER, endoplasmic reticulum; PLD, phospholipase D; LPCAT, lysophosphatidylcholine:acyl-CoA acyl transferase.

REFERENCES


