Two Stories About Plasmalogens

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April 1, 2019

Today I am gonna tell you a couple of stories about plasmalogens. First story is about plasmalogens as regulators on innate immune reactions via priming of macrophages, and the second is about plasmalogens as regulators of the phagocytic process. But before getting into those molecular details that we chemists and biochemists like so much, let's start from the beginning: what is a plasmalogen? Where does its unusual name come from? What does it do? Is a plasmalogen good or bad? A plasmalogen is just a glycerophospholipid with an unusual vinyl ether bond, instead of the usual acyl, in the sn-1 position of the glycerol backbone…

University of the Basque Country – April 1-5, 2019.

Story One: Plasmalogen priming effects on arachidonic acid mobilization.

Slide 1. Lipopolysaccharide priming of mouse peritoneal macrophages for enhanced arachidonic acid release in response to zymosan. Kinetics of arachidonic acid release; effect of lipopolysaccharide concentration; effect of zymosan concentration; effect of the duration of preincubation of the cells with lipopolysaccharide.

Slide 2. Arachidonic acid-containing phospholipid species in macrophages. Ethanolamine plasmalogen species, diacyl PC species, and stearoyl phosphatidylinositol (PI) were the major AA-containing species. Treatment of the cells with LPS did not induce any appreciable change in the content or species distribution of AA. When unprimed cells were stimulated with yeast-derived zymosan particles, marked decreases in the content of cellular AA-containing phospholipids were appreciated. All major AA-containing PC species plus PI (18:0/20:4) contributed to this release. On the opposite side, no PE species significantly changed its AA content.

Slide 3. Effect of pyrrophenone on phospholipid deacylation reactions in macrophages. LPS priming facilitates the hydrolysis of plasmalogen PE species after zymosan stimulation. All these decreases were prevented if the zymosan incubations included the well-established $cPLA_2\alpha$ inhibitor pyrrophenone, thus confirming the general role that $cPLA_2\alpha$ plays in macrophage AA release.

Slide 4. Lysophospholipid molecular species generated by activated macrophages. Significant increases in a number of lysoPC and lysoPI species were detected, and their levels were further increased in the LPS-primed cells. Regarding lysoPE species, significant increases of ethanolamine lysophospholipids were observed only in the LPS-primed cells zymosan-stimulated, and involved only the plasmalogen forms

Slide 5. Characterization of plasmalogen-deficient RAW.12 cells. Despite the considerable absence of ether phospholipids in RAW.12 cells (including ethanolamine pasmalogens and alkyl-PC species), their AA content was found to be the same as that of native RAW264.7 cells, as quantified by gas chromatography/mass

spectrometry. The distribution of AA between phospholipid classes was also preserved in the RAW.12 cell compared to native RAW264.7, because of a compensatory elevation of the levels of AA in diacyl species.

Slide 6. Phospholipid molecular species that incorporate exogenous AA. Most of the AA incorporated into PC species, with minor amounts incorporating into PI species. No PE species incorporated significant amounts of fatty acid and, importantly, the profile of AA incorporation into PC and PI was the same, whether the cells were primed with LPS or not.

Slide 7. Phospholipid arachidonic acid remodeling in macrophages. A low but significant amount of label was found in the plasmalogen species PE(P-16:0/[^{2}H]AA) after a 1-h incubation with zymosan, confirming the involvement of CoA-IT in the stimulated cells. Similarly, accumulation of label was also found in the ether phospholipid species PC(O-16:0/[^{2}H]AA), which is also a preferred acceptor for CoA-IT-dependent transacylation reactions. Neither of these species appeared in the LPS-primed zymosan-stimulated cells, strongly indicating that the CoA-IT pathway is blunted by LPS priming.

Slide 8. Interactions between CoA-dependent acyltransferases, CoA-independent transacylase, and phospholipase A_2 in regulating arachidonic acid release in macrophages.

Story Two. Regulation of phagocytosis by plasmalogens.

Slide 1. Comparison between RAW 264.7 and plasmalogen-deficient RAW.108 cells. Phagocytosis of opsonized zymosan particles was significantly impaired in the ethanolamine plasmalogen-deficient RAW.108 cells

Slide 2. Ethanolamine plasmalogen content of RAW 264.7 and RAW.108 cells. Comparative lipidomic analyses of wildtype cells versus RAW.108 cells by mass spectrometry confirmed the practically complete absence of plasmalogens in the latter.

Slide 3. Incubation of RAW.108 cells with lysoPlsEtn increases plasmalogen content. Treatment with lysoPlsEtn significantly elevated the amount of plasmalogen in the RAW.108 cells, especially that containing arachidonic acid, which increased to levels close to 60–70% of those of normal RAW 264.7 cells.

Slide 4. Effect of lysoPlsEtn on the phagocytosis of opsonized zymosan by RAW 264.7 and plasmalogen-deficient RAW.108 cells. Assays utilizing cells incubated with lysoPlsEtn showed that this simple treatment was enough to increase the capacity of RAW.108 cells to phagocytize the opsonized zymosan.

Slide 5. Effect of lysoPlsEtn on the phagocytosis of different targets by RAW 264.7 and plasmalogen-deficient RAW.108 cells. Incubation of macrophages with lysoPlsEtn did significantly increase the capacity of RAW.108 cells to phagocytize both unopsonized latex beads and apoptotic cells; however, the extent of the effect was smaller than that found when opsonized zymosan was used as a target.

Slide 6. Effect of lysoPlsEtn on membrane rafts in RAW264.7 and plasmalogen-deficient RAW.108 cells. Treating either RAW 264.7 cells or RAW.108 cells with lysoPlsEtn, but not lysoPtdEtn, increased both the number and size of lipid rafts in the two types of cells similarly. Collectively, these results suggest that enriching the cells with ethanolamine plasmalogen selectively increases both the number and size of lipid rafts on the plasma membrane, and the overall phagocytic capacity of the cells.

Slide 7. Analysis of membrane fluidity in RAW 264.7 and plasmalogen-deficient RAW.108 cells. The membrane mobile fraction of RAW264.7 cells remains basically unaltered after treating the cells with lysoPlsEtn, which was to be expected since, as indicated previously, treating these cells with exogenous lysoPlsEtn only slightly increases the amount of cellular ethanolamine plasmalogen of the cells.

Slide 8. Effect of lysophospholipids on zymosan-induced signaling. LysoPlsEtn treatment of the RAW.108 cells

significantly increased the phosphorylation activation of the extracellular-regulated kinases p44 and p42 by opzonized zymosan.

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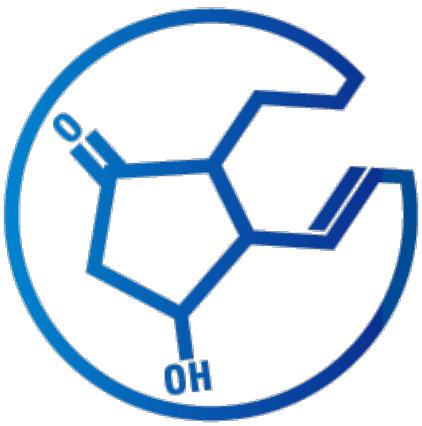

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