

# Procesos de remodelación de ácido araquidónico entre fosfolípidos durante la estimulación fagocítica de macrófagos peritoneales de ratón

Jesús Balsinde, et al.

*Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC),  
47003 Valladolid, Spain, and*

*Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM),  
28029 Madrid, Spain*

July 29, 2020

Los macrófagos contienen grandes cantidades de ácido araquidónico (AA), que se distribuye de manera asimétrica entre los fosfolípidos de la membrana. Esto se debe en gran parte a la acción de la transacilasa independiente de coenzima A (CoA-IT), que transfiere el AA principalmente de diacil-fosfolípidos de colina a fosfolípidos que contienen etanolamina. En este trabajo se han analizado comparativamente los cambios en glicerofosfolípidos que ocurren cuando se libera AA de macrófagos peritoneales de ratón en respuesta a zimosán o zimosán opsonizado (OpZ). Estos dos estímulos fagocíticos promueven la movilización dependiente de fosfolipasa A<sub>2</sub> del AA a través de la activación de receptores de superficie distintos. Los estudios por espectrometría de masas revelaron disminuciones significativas en los niveles de todas las especies moleculares mayoritarias de fosfolípidos de colina y una especie de fosfatidilinositol. Es importante destacar que, si bien no se detectaron cambios en las especies de fosfolípidos de etanolamina durante la estimulación con zimosan, sí se observaron disminuciones significativas en estas especies cuando se utilizó OpZ. Los análisis de la remodelación de AA mediada por CoA-IT revelaron que el proceso se produce más rápidamente en células estimuladas por zimosan en comparación con las células estimuladas por OpZ. La inhibición farmacológica de CoA-IT inhibió fuertemente la liberación de AA en respuesta a zimosán, pero sólo tuvo un efecto moderado en la respuesta mediada por OpZ. Estos resultados sugieren un papel hasta ahora no descrito, dependiente de receptor, para las reacciones de remodelación de AA independientes de CoA en la modulación de la producción de eicosanoides por los macrófagos. Estos datos ayudan a definir nuevos objetivos dentro de la vía de remodelación de AA con posible valor potencial para el control de la formación de mediadores de lípidos.

Financiación: Ministerio de Economía, Industria y Competitividad (SAF2016-80883-R)

## REFERENCES

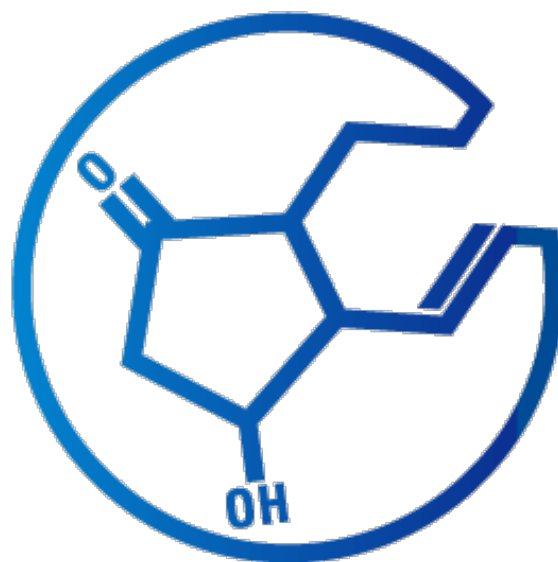
1. Astudillo, A. M., D. Balgoma, M. A. Balboa, and J. Balsinde. 2012. Dynamics of arachidonic acid mobilization by inflammatory cells. *Biochim. Biophys. Acta* 1821: 249–256.
2. Pérez-Chacón, G., A. M. Astudillo, D. Balgoma, M. A. Balboa, and J. Balsinde. 2009. Control of free arachidonic acid levels by phospholipases A<sub>2</sub> and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103–1113.
3. Gil-de-Gómez, L., A. M. Astudillo, C. Meana, J. M. Rubio, C. Guijas, M. A. Balboa, and J. Balsinde. 2013. A phosphatidylinositol species acutely generated by activated macrophages regulates innate immune responses. *J. Immunol.* 190: 5169–5177.

4. Gil-de-Gómez, L., A. M. Astudillo, C. Guijas, V. Magrioti, G. Kokotos, M. A. Balboa, and J. Balsinde. 2014. Cytosolic group IVA and calcium-independent group VIA phospholipase A<sub>2</sub>s act on distinct phospholipid pools in zymosan-stimulated mouse peritoneal macrophages. *J. Immunol.* 192: 752–762.
5. Monge, P., A. Garrido, J.M. Rubio, V. Magrioti, G. Kokotos, M.A. Balboa, and J. Balsinde, J. 2020. The contribution of cytosolic group IVA and calcium-independent group VIA phospholipase A<sub>2</sub>s to adrenergic acid mobilization in murine macrophages. *Biomolecules* 10: 542.
6. Rubio, J. M., J. P. Rodríguez, L. Gil-de-Gómez, C. Guijas, M. A. Balboa, and J. Balsinde. 2015. Group V secreted phospholipase A<sub>2</sub> is up-regulated by interleukin-4 in human macrophages and mediates phagocytosis via hydrolysis of ethanolamine phospholipids. *J. Immunol.* 194: 3327–3339.
7. Gil-de-Gómez, L., A. M. Astudillo, P. Lebrero, M. A. Balboa, and J. Balsinde. 2017. Essential role for ethanolamine plasmalogen hydrolysis in bacterial lipopolysaccharide priming of macrophages for enhanced arachidonic acid release. *Front. Immunol.* 8: 1251.
8. Rubio, J. M., A. M. Astudillo, J. Casas, M. A. Balboa, and J. Balsinde. 2018. Regulation of phagocytosis in macrophages by membrane ethanolamine plasmalogens. *Front. Immunol.* 9: 1723.
9. Pérez, R., X. Matabosch, A. Llebaria, M.A. Balboa, and J. Balsinde. 2006. Blockade of arachidonic acid incorporation into phospholipids induces apoptosis in U937 promonocytic cells. *J. Lipid Res.* 47: 484–491.
10. Balsinde, J., B. Fernández, and E. Diez. 1990. Regulation of arachidonic acid release in mouse peritoneal macrophages. The role of extracellular calcium and protein kinase C. *J. Immunol.* 144: 4298–4304.
11. Balsinde, J., M.A. Balboa, and E.A. Dennis. 2000. Identification of a third pathway for arachidonic acid mobilization and prostaglandin production in activated P388D<sub>1</sub> macrophage-like cells. *J. Biol. Chem.* 275: 22544–22549.
12. Casas, J., C. Meana, E. Esquinas, M. Valdearcos, J. Pindado, J. Balsinde, and M.A. Balboa. 2009. Requirement of JNK-mediated phosphorylation for translocation of group IVA phospholipase A<sub>2</sub> to phagosomes in human macrophages. *J. Immunol.* 183: 2767–2774.
13. Balboa, M. A., Y. Sáez, and J. Balsinde. 2003. Calcium-independent phospholipase A<sub>2</sub> is required for lysozyme secretion in U937 promonocytes. *J. Immunol.* 170: 5276–5280.
14. Balboa, M. A., R. Pérez, and J. Balsinde. 2003. Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A<sub>2</sub> is regulated by cytosolic phospholipase A<sub>2</sub>-derived hydroperoxyeicosatetraenoic acid. *J. Immunol.* 171: 989–994.
15. Balsinde, J., M.A. Balboa, P.A. Insel, and E.A. Dennis. 1997. Differential regulation of phospholipase D and phospholipase A<sub>2</sub> by protein kinase C in P388D<sub>1</sub> macrophages. *Biochem. J.* 321: 805–809.
16. Ruipérez, V., A. M. Astudillo, M. A. Balboa, and J. Balsinde. 2009. Coordinate regulation of TLR-mediated arachidonic acid mobilization in macrophages by group IVA and group V phospholipase A<sub>2</sub>s. *J. Immunol.* 182: 3877–3883.
17. Casas, J., M.A. Gijón, A.G. Vigo, M.S. Crespo, J. Balsinde, and M.A. Balboa. 2006. Phosphatidylinositol 4,5-bisphosphate anchors cytosolic group IVA phospholipase A<sub>2</sub> to perinuclear membranes and decreases its calcium requirement for translocation in live cells. *Mol. Biol. Cell* 17: 155-162.
18. Pindado, J., J. Balsinde, and M. A. Balboa. 2007. TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase 2/cyclooxygenase-2 pathway. *J. Immunol.* 179: 4821–4828.
19. Balboa, M. A., J. Balsinde, and E. A. Dennis. 1998. Involvement of phosphatidate phosphohydrolase in arachidonic acid mobilization in human amnionic WISH cells. *J. Biol. Chem.* 273: 7684-7690.
20. Balboa, M. A., J. Balsinde, D. A. Dillon, G. M. Carman, and E. A. Dennis. 1999. Proinflammatory macrophage-activating properties of the novel phospholipid diacylglycerol pyrophosphate. *J. Biol. Chem.* 274: 522–526.
21. Balsinde, J., M. A. Balboa, S. Yedgar, and E. A. Dennis. 2000. Group V phospholipase A<sub>2</sub>-mediated oleic acid mobilization in lipopolysaccharide-stimulated P388D<sub>1</sub> macrophages. *J. Biol. Chem.* 275: 4783–4786.

22. Balsinde, J. 2002. Roles of various phospholipases A<sub>2</sub> in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J.* 364: 695–702.
23. Diez, E., J. Balsinde, M. Aracil, and A. Schüller. 1987. Ethanol induces release of arachidonic acid but not synthesis of eicosanoids in mouse peritoneal macrophages. *Biochim. Biophys. Acta* 921: 82–89.
24. Guijas, C., G. Pérez-Chacón, A. M. Astudillo, J. M. Rubio, L. Gil-de-Gómez, M. A. Balboa, and J. Balsinde. 2012. Simultaneous activation of p38 and JNK by arachidonic acid stimulates the cytosolic phospholipase A<sub>2</sub>-dependent synthesis of lipid droplets in human monocytes. *J. Lipid Res.* 53: 2343–2354.
25. Guijas, C., C. Meana, A. M. Astudillo, M. A. Balboa, and J. Balsinde. 2016. Foamy monocytes are enriched in cis-7-hexadecenoic fatty acid (16:1n-9), a possible biomarker for early detection of cardiovascular disease. *Cell Chem. Biol.* 23: 689–699.
26. Rodríguez, J. P., C. Guijas, A. M. Astudillo, J. M. Rubio, M. A. Balboa, and J. Balsinde. 2019. Sequestration of 9-hydroxystearic acid in FAHFA (fatty acid esters of hydroxy fatty acids) as a protective mechanism for colon carcinoma cells to avoid apoptotic cell death. *Cancers* 11: 524.
27. Balgoma, D., O. Montero, M.A. Balboa, and J. Balsinde. 2008. Calcium-independent phospholipase A<sub>2</sub>-mediated formation of 1,2-diarachidonoylglycerophosphoinositol in monocytes. *FEBS J.* 275: 6180–6191.
28. Balgoma, D., A. M. Astudillo, G. Pérez-Chacón, O. Montero, M. A. Balboa, and J. Balsinde. 2010. Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids. *J. Immunol.* 184: 3857–3865.
29. Astudillo, A. M., G. Pérez-Chacón, C. Meana, D. Balgoma, A. Pol, M. A. del Pozo, M. A. Balboa, and J. Balsinde. 2011. Altered arachidonate distribution in macrophages from caveolin-1 null mice leading to reduced eicosanoid synthesis. *J. Biol. Chem.* 286: 35299–35307.
30. Valdearcos, M., E. Esquinas, C. Meana, L. Gil-de-Gómez, C. Guijas, J. Balsinde, and M. A. Balboa. 2011. Subcellular localization and role of lipin-1 in human macrophages. *J. Immunol.* 186: 6004–6013.
31. Guijas, C., A. M. Astudillo, L. Gil-de-Gómez, J. M. Rubio, M. A. Balboa, and J. Balsinde. 2012. Phospholipid sources for adrenic acid mobilization in RAW 264.7 macrophages: comparison with arachidonic acid. *Biochim. Biophys. Acta* 1821: 1386–1393.
32. Lebrero, P., A.M. Astudillo, J.M. Rubio, L. Fernández-Caballero, G. Kokotos, M.A. Balboa, and J. Balsinde. 2019. Cellular plasmalogen content does not influence arachidonic acid levels or distribution in macrophages: a role for cytosolic phospholipase A<sub>2</sub>γ in phospholipid remodeling. *Cells* 8: 799.
33. Rodríguez, J.P., E. Leiguez, C. Guijas, B. Lomonte, J.M. Gutiérrez, C. Teixeira, M.A. Balboa, and J. Balsinde. 2020. A lipidomic perspective of the action of group IIA secreted phospholipase A<sub>2</sub> on human monocytes: lipid droplet biogenesis and activation of cytosolic phospholipase A<sub>2</sub>α. *Biomolecules* 10: 891.
34. Balsinde, J., B. Fernández, J.A. Solís-Herruzo, and E. Diez. 1992. Pathways for arachidonic acid mobilization in zymosan-stimulated mouse peritoneal macrophages. *Biochim. Biophys. Acta* 1136: 75–82.
35. Pérez-Chacón, G., A.M. Astudillo, V. Ruipérez, M.A. Balboa, and J. Balsinde. 2010. Signaling role for lysophosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. *J. Immunol.* 184: 1071–1078.
36. Guijas, C., M.A. Bermúdez, C. Meana, A.M. Astudillo, L. Pereira, L. Fernández-Caballero, M.A. Balboa, and J. Balsinde. 2019. Neutral lipids are not a source of arachidonic acid for lipid mediator signaling in human foamy monocytes. *Cells* 8: 941.
37. Balboa, M. A., and J. Balsinde. 2002. Involvement of calcium-independent phospholipase A<sub>2</sub> in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem.* 277: 40384–40389.
38. Astudillo, A. M., M. A. Balboa, and J. Balsinde. 2019. Selectivity of phospholipid hydrolysis by phospholipase A<sub>2</sub> enzymes in activated cells leading to polyunsaturated fatty acid mobilization.

*Biochim. Biophys. Acta* 1864: 772–783.

39. Astudillo, A. M., G. Pérez-Chacón, D. Balgoma, L. Gil-de-Gómez, V. Ruipérez, C. Guijas, M. A. Balboa, and J. Balsinde. 2011. Influence of cellular arachidonic acid levels on phospholipid remodeling and CoA-independent transacylase activity in human monocytes and U937 cells. *Biochim. Biophys. Acta* 1811: 97–103.
40. Astudillo, A. M., C. Meana, C. Guijas, L. Pereira, R. Lebrero, M. A. Balboa, and J. Balsinde. 2018. Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. *J. Lipid Res.* 59: 237–249.
41. Guijas, C., J. P. Rodríguez, J. M. Rubio, M. A. Balboa, and J. Balsinde. 2014. Phospholipase A<sub>2</sub> regulation of lipid droplet formation. *Biochim. Biophys. Acta* 1841: 1661–1671.
42. Balgoma, D., O. Montero, M. A. Balboa, and J. Balsinde. 2010. Lipidomic approaches to the study of phospholipase A<sub>2</sub>-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 92: 645–650.



**THE EICOSANOID  
RESEARCH DIVISION**  
VALLADOLID