

Incorporación, remodelación y movilización de ácido docosahexaenoico en 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*

November 7, 2023

En este trabajo se ha investigado la incorporación de ácido docosahexaenoico (DHA) en macrófagos peritoneales residentes en ratones y su redistribución dentro de las diversas clases de fosfolípidos. Los glicerofosfolípidos (PC) de colina se comportaron como los principales aceptores iniciales de DHA. La incubación prolongada con el ácido graso resultó en la transferencia de DHA de PC a glicerofosfolípidos de etanolamina (PE), lo que refleja remodelación de fosfolípidos. Este proceso dio como resultado que las células contuvieran cantidades similares de DHA en PC y PE en estado de reposo. Los análisis lipidómicos basados en espectrometría de masas de especies moleculares de fosfolípidos indicaron una marcada abundancia de DHA en los fosfolípidos con enlaces éter. La estimulación de los macrófagos con zimosán dio como resultado disminuciones significativas de los niveles de todas las especies de PC y PI que contienen DHA; sin embargo, no se encontró que ninguna especie molecular de PE o PS disminuyera. Por el contrario, se encontró que los niveles de una especie inusual que contiene DHA, a saber, PI (20:4/22:6), que apenas estaba presente en las células en reposo, aumentaban notablemente bajo la estimulación con zimosano. Los niveles de este fosfolípido también aumentaron significativamente cuando se utilizó el ionóforo de calcio A23187 o el factor activador de plaquetas en lugar de zimosan para estimular los macrófagos. El estudio de la ruta involucrada en la síntesis de PI(20:4/22:6) sugiere que esta especie se produce mediante reacciones de desacilación/reacilación. Estos resultados definen los aumentos en PI(20:4/22:6) como un nuevo marcador metabólico lipídico de la activación de macrófagos y proporcionan información novedosa para comprender la regulación del recambio de ácidos grasos en los fosfolípidos de macrófagos activados.

Financiación: Ministerio de Ciencia e Innovación (PID2019-105989RB-I00)
Junta de Castilla y León (CSI141P20)

REFERENCES

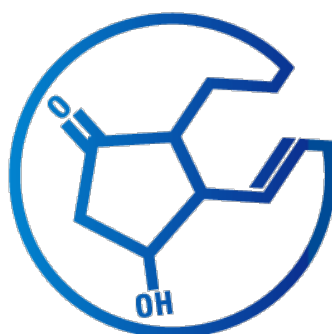
1. Astudillo, A.M., C. Meana, C. Guijas, L. Pereira, P. Lebrero, M.A. Balboa, and J. Balsinde. 2018. Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. *J. Lipid Res.* 59: 237–249.
2. Pérez-Chacón, G., A.M. Astudillo, D. Balgoma, M.A. Balboa, and J. Balsinde. 2009. Control of free arachidonic acid levels by phospholipases A₂ and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103–1113.
3. 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.
4. Astudillo, A.M., M.A. Balboa, and J. Balsinde. 2019. Selectivity of phospholipid hydrolysis by phospholipase A₂ enzymes in activated cells leading to polyunsaturated fatty acid mobilization. *Biochim. Biophys. Acta*

1864: 772–783.

5. Astudillo, A.M., M.A. Balboa, and J. Balsinde. 2023. Compartmentalized regulation of lipid signaling in oxidative stress and inflammation: Plasmalogens, oxidized lipids and ferroptosis as new paradigms of bioactive lipid research. *Prog. Lipid Res.* 89: 101207.
6. 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.
7. Rubio, J.M., J.P. Rodríguez, L. Gil-de-Gómez, C. Guijas, M.A. Balboa, and J. Balsinde. 2015. Group V secreted phospholipase A₂ is up-regulated by interleukin-4 in human macrophages and mediates phagocytosis via hydrolysis of ethanolamine phospholipids. *J. Immunol.* 194: 3327–3339.
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. 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 A₂γ in phospholipid remodeling. *Cells* 8: 799.
10. 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 A₂s act on distinct phospholipid pools in zymosan-stimulated mouse peritoneal macrophages. *J. Immunol.* 192: 752–762.
11. Astudillo, A.M., J.P. Rodríguez, C. Guijas, J.M. Rubio, M.A. Balboa, M.A., and J. Balsinde. 2021. Choline glycerophospholipid-derived prostaglandins attenuate TNFα gene expression in macrophages via a cPLA₂α/COX-1 pathway. *Cells* 10: 447.
12. Guijas, C., J.P. Rodríguez, J.M. Rubio, M.A. Balboa, and J. Balsinde. 2014. Phospholipase A₂ regulation of lipid droplet formation. *Biochim. Biophys. Acta* 1841: 1661–1671.
13. 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.
14. 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 A₂s. *J. Immunol.* 182: 3877–3883.
15. 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.
16. Balsinde, J., M.A. Balboa, and E.A. Dennis. 2000. Identification of a third pathway for arachidonic acid mobilization and prostaglandin production in activated P388D₁ macrophage-like cells. *J. Biol. Chem.* 275: 22544–22549.
17. Balboa, M.A., Y. Sáez, and J. Balsinde. 2003. Calcium-independent phospholipase A₂ is required for lysozyme secretion in U937 promonocytes. *J. Immunol.* 170: 5276–5280.
18. Balboa, M.A., R. Pérez, and J. Balsinde. 2003. Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A₂ is regulated by cytosolic phospholipase A₂-derived hydroperoxyeicosatetraenoic acid. *J. Immunol.* 171: 989–994.
19. Balsinde, J., M. A. Balboa, S. Yedgar, and E. A. Dennis. 2000. Group V phospholipase A₂-mediated oleic acid mobilization in lipopolysaccharide-stimulated P388D₁ macrophages. *J. Biol. Chem.* 275: 4783–4786.
20. Balsinde, J., M.A. Balboa, P.A. Insel, and E.A. Dennis. 1997. Differential regulation of phospholipase D and phospholipase A₂ by protein kinase C in P388D₁ macrophages. *Biochem. J.* 321: 805–809.
21. 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.
22. Balsinde, J. 2002. Roles of various phospholipases A₂ in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J.* 364: 695–702.
23. Astudillo, A.M., C. Meana, M.A. Bermúdez, A. Pérez-Encabo, M.A. Balboa, and J. Balsinde. 2020. Release

- of anti-inflammatory palmitoleic acid and its positional isomers by mouse peritoneal macrophages. *Biomedicines* 8: 480.
24. Monge, P.; Garrido, A.; Rubio, J.M.; Magrioti, V.; Kokotos, G.; Balboa, M.A.; Balsinde, J. The contribution of cytosolic group IVA and calcium-independent group VIA phospholipase A₂s to adrenergic acid mobilization in murine macrophages. *Biomolecules* **2020**, *10*, 542.
 25. 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 A₂-dependent synthesis of lipid droplets in human monocytes. *J. Lipid Res.* *53*: 2343–2354.
 26. 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.
 27. 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.
 28. 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.
 29. 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.
 30. Balboa, M.A., and J. Balsinde. 2002. Involvement of calcium-independent phospholipase A₂ in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem.* *277*: 40384–40389.
 31. 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.
 32. 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.
 33. Gil-de-Gómez, L., P. Monge, J.P. Rodríguez, A.M. Astudillo, M.A. Balboa, and J. Balsinde. 2020. Phospholipid arachidonic acid remodeling during phagocytosis in mouse peritoneal macrophages. *Biomedicines* *8*: 274.
 34. Balgoma, D., O. Montero, M.A. Balboa, and J. Balsinde. 2008. Calcium-independent phospholipase A₂-mediated formation of 1,2-diarachidonoylglycerophosphoinositol in monocytes. *FEBS J.* *275*: 6180–6191.
 35. 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 A₂ to perinuclear membranes and decreases its calcium requirement for translocation in live cells. *Mol. Biol. Cell* *17*: 155–162.
 36. Bermúdez M.A., M.A. Balboa, and J. Balsinde. 2021. Lipid droplets, phospholipase A₂, arachidonic acid, and atherosclerosis. *Biomedicines* *9*: 1891.
 37. Bermúdez, M.A., L. Pereira, C. Fraile, L. Valerio, M.A. Balboa, and J. Balsinde. 2022. Roles of palmitoleic acid and its positional isomers, hypogeic and sapienic acids, in inflammation, metabolic diseases and cancer. *Cells* *11*: 2146.
 38. Bermúdez, M.A., J.M. Rubio, M.A. Balboa, and Balsinde, J. 2022. Differential mobilization of the phospholipid and triacylglycerol pools of arachidonic acid in murine macrophages. *Biomolecules* *12*: 1851.
 39. Balboa, M.A., J. Balsinde, and E. A. Dennis. 2000. Phosphorylation of cytosolic group IV phospholipase A₂

- is necessary but not sufficient for arachidonic acid release in P388D₁ macrophages. *Biochem. Biophys. Res. Commun.* 267: 145–148.
40. Balboa, M.A., J. Balsinde, C.A. Johnson, and E.A. Dennis. 1999. Regulation of arachidonic acid mobilization in lipopolysaccharide-activated P388D₁ macrophages by adenosine triphosphate. *J. Biol. Chem.* 274: 36764–36768.
41. Balsinde, J., and E.A. Dennis. 1996. The incorporation of arachidonic acid into triacylglycerol in P388D₁ macrophage-like cells. *Eur. J. Biochem.* 235: 480–485.
42. Balsinde, J., E. Diez, B. Fernández, and F. Mollinedo. 1989. Biochemical characterization of phospholipase D activity from human neutrophils. *Eur. J. Biochem.* 186: 717–724.
43. 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.
44. 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.
45. Balboa, M.A., and J. Balsinde. 2021. Phospholipases: from structure to biological function. *Biomolecules* 11: 428.



**THE EICOSANOID
RESEARCH DIVISION**
VALLADOLID