Innate Immunity, Inflammation, Lipid Signaling, and Lipidomics

Jesús Balsinde

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

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This a transcription of the lecture presented on Friday May 18, 2012 at the University of Barcelona, Bellvitge Campus, main Conference Hall (Slide 1) [1]

Lipids have traditionally been considered to exert two main missions in Physiology: to serve as a source of energy from nutrients and as structural components of cell membranes (Slide 2 - Classical View of Lipids). However this view has changed enormously, for good, in the last 30-40 years with the discovery that lipids are key in signaling (Slide 3 - Current View of Lipids). Intracellular signaling systems that connect external stimuli with the execution of specific responses have been discovered that utilize lipids as second mesengers. Three of them are highlighted in this slide, those that utilize phospholipase C, sphingomyelinase and phospholipase A_2 as signal amplifiers. The latter uses multiple messengers, both intra and extracellular and, although present in all cells, it is of special interest in cells of the innate immunity [2-4]. Eicosanoids play a large number of roles in the human body, by regulating a host of physiological actions which are shown in the figure (Slide 4 - Role of Eicosanoids in the Human Body). The role of eicosanoids inflammation is illustrated by the famous cartoon by Lawrence et al in Nature Reviews in Immunology (vol 2, pp 787-795, 2002), where eicosanoids are responsible for all four cardinal signs of inflammation (Slide 5 - Untitled). The importance of lipids and inflammation is such that it has even come to affect the evolution of the human race. If two and a half million years have been needed to get to the homo sapiens, in just thirty years we are witnessing the emergence of a new step in human evolution; the homo obesus (Slide 6 - Lipids, Inflammation and Evolution). You can see a happy obese guy in the next slide (Slide 7 - The Risks of Eating Too Much Fat). His name as you all know is Obelix. He does not know, but his risk of suffering a stroke is three times greater than that of a normal person; his risk of being hypertensive is also three times higher; his risk of suffering from a cardiovascular problem is four times greater and his risk of developing type 2 diabetes is three times higher. All of these diseases have one thing in common: disregulated lipid metabolism, hence if we want to cure these diseases we have to know what these lipids are and what they do. This leads to the concept of lipidomics as ennunciated by Ed Dennis (Slide 8 - What Is Lipidomics?) and the "official" definition of the Europeans (Slide 9 - What Is Lipidomics?). The tool of preference for use in lipidomics is mass spectrometry (Slide 10 - Lipidomics: Mass Spectrometry). In our lab in Valladolid we have been doing lipidomics for a long time, always under the general subject of Lipid Signaling in Innate Immunity and Inflammation. Our main focus is all about arachidonic acid (Slide 11 - Lipidomics in Valladolid), which implies not only the fatty acid and its oxygenated metabolites, but also other related fatty acids such as e.g. adrenic acid and the glycerophospholipids that store arachidonate in cells [1, 5-11]. Yes, arachidonate is not found as a free fatty acid in cells but is rather found esterified in membrane phospholipids, particularly PC, PE and PI (Slide 12 – Role of Phospholipase A_2 in Arachidonic Acid Release) [2,4,11], Free AA in cells depends on a deacylation/reacylation cycle controlled by phospholipases A_2, CoA-dependent acyltransferases and CoA-independent transacylases (Slide 13 – Free AA levels depend on a deacylation/reacylation cycle) (Slide 14 – AA Incorporation Into and Remodeling Between Phospholipids) [3, 8, 13, 14].

We start the presentation of results from our lab with the work of David Bálgoma concerning the identification of lipid markers of activation (Slide 15 - Lipidomics as a Useful Approach to Identify Markers of Activation) [5,6]. The procedure is described in detail in the next slide and involves the use of pyrrophenone, an inhibitor of cPLA$_2\alpha$ [15,16], to prevent release of AA from phospholipids and thus serve as a control of these experiments (Slide 16 – Markers of Activation Revealed by Lipidomic Profiling of Arachidonic Acid-Containing Phospholipids). But before we get to the results we have to explain briefly first how phospholipid molecular species are named (Slide 17 – Glycerophospholipids - Nomenclature). PC(18:0/20:4) is used as an example, and the special case of ether bonds in position sn-1 is highlighted, since in phagocytic cells it is very frequent that if AA in position 2, there is an ether in position 1. The next slide shows the 'arachidonome' of human monocytes classified by phospholipid class (Slide 18 - AA-Containing Phospholipids in Unstimulated Human Monocytes). The time-courses of different kinds of phospholipids, are displayed: PC (Slide 19 - Time - dependent Changes of Major AA-containing PC Species After Zymosan Stimulation), PI (Slide 20 - Time - dependent Changes of Major AA-containing PI Species After Zymosan Stimulation), and PE (Slide 21 - Time - dependent Changes of Major AA-containing PE Species After Zymosan Stimulation) and the appearance of species showing unusual behavior is emphasized. The effect of triacsin C, and inhibitor of acyl-CoA synthetases on the production of these unusual lipids is shown (Slide 22 – The Role of CoA-dependent Acyl Transferases). Production of PC(20:4/20:4) and PI(20:4/20:4) but not of PE(16:1/20:4) was inhibited by triacsin C. Next, the effect of other stimuli on the productioon of these lipids is shown (Slide 23 – What About Other Stimuli?)PE(16:1/20:4) appears only with zymosan. With these data, the first part of the talk is closed with a summary that suggests lipidomics as a useful approach to identify specific molecular markers in resting versus activated cells (Slide 24 – Conclusions)

We move to the second part of the lecture, focused on the use of lipidomics to identify new proteins involved in activation (Slide 25 – Lipidomics as a useful approach to identify new protein effectors involved in inflammation). In our studies in eicosanoid production in macrophages, we became interested in caveolin-1, a fatty acid-binding protein that is present in caveolae (Slide 26 – Caveolae and Caveolin-1). Caveolin-1 K.O. mice are available (Slide 27 - Caveolin-1 KO Mice). Macrophages from these mice incorporate AA into lipids to a greater extent that normal cells (Slide 28 - AA Incorporation Into Phospholipids). The individual species that capture this AA by LC/MS are shown afterward (Slide 29 - AA Incorporation Into Phospholipids) [9]. Do these changes persist over time? It becomes necessary to analyze the species under equilibrium conditions (Slide 30 - Untitled). Phospholipid fatty acid content is measured by GC/MS and no chages are appreciated (Slide 31 - Phospholipid Fatty Acid Content) [14]. Subsequently the distribution in phospholipids of endogenous AA by LC/MS/MS is shown, and significant variations are appreciated (Slide 32 - AA Containing Phospholipids). The next slide shows the data grouped by phospholipid classes (Slide 33 - Equilibrium). The data suggest variations in the CoA-independent transacylase, so its activity was measured and an increase was found in the KO cells (Slide 34 - CoA-IT Activity Is Augmented in Homogenates from Cav-1-deficient Cells) [9]. Here it is note again that AA levels depend on a deacylation/reacylation cycle (Slide 35 – Free AA Levels Depend on a Deacylation/Reacylation Cycle). Thus, the release of AA stimulated by zymosan, a prototypical stimulus of macrophages [17-20] is shown next, which is decreased in the cav-1-deficient cells (Slide 36 – AA Mobilization in Control and Cav-1-deficient Mice). Eicosanoids are also decreased (Slide 37 - Eicosanoid Production in Control and Cav-1-deficient Mice) [9]. The final summary slide is explained next, indicating with arrows the chnges that take place when caveolin is absent (Slide 38 – Caveolin-1). Preliminary data are shown of a peritonitis model that is consistent with the previous findings (Slide 39 - Infiltration of neutrophils in zimosan-induced peritonitis model). The closing slide follows, where the final splash message is displayed: lipidomics is good to study lipid markers of activation and to find new protein effectors (Slide 40 - The Lipidomics Approach to Cellular Signaling). Thank you very much for your attention. (Slide 41 - Acknowlegments). (Unused slides from refs. 21-25).

REFERENCES

1. 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.

2. Balsinde, J., M. V. Winstead, and E. A. Dennis. 2002. Phospholipase A_2 regulation of arachidonic acid mobilization. *FEBS Lett*. 531: 2-6.

3. Pérez-Chacón, G., A. M. Astudillo, D. Balgoma, M. A. Balboa, and J. Balsinde. 2009. Control of free arachidonic acid levels by phospholipases A_2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103–1113.

4. 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.

5. Balgoma, D., O. Montero, M. A. Balboa, and J. Balsinde. 2008. Calcium-independent phospholipase A_2-mediated formation of 1,2-diarachidonoyl-glycerophosphoinositol in human monocytes. *FEBS J.* 275: 6180–6191.

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. Balgoma, D., O. Montero, M. A. Balboa, and J. Balsinde. 2010. Lipidomic approaches to the study of phospholipase A_2-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 92: 645-650.

8. 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.

9. 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.

10. 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.

11. 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_2-dependent synthesis of lipid droplets in human monocytes. *J. Lipid Res*. 53: 2343–2354.

12. Balsinde, J., M. A. Balboa, P. A. Insel, and E. A. Dennis. 1999. Regulation and inhibition of phospholipase A_2. *Annu. Rev. Pharmacol. Toxicol*. 39: 175-189.

13. Balsinde, J. 2002. Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J*. 364: 695-702.

14. 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.

15. Balboa, M. A., and J. Balsinde. 2002. Involvement of calcium-independent phospholipase A_2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem*. 277: 40384–40389.

16. Balboa, M. A., R. Pérez, and J. Balsinde. 2008. Calcium-independent phospholipase A_2 mediates proliferation of human promonocytic U937 cells. *FEBS J.* 275: 1915–1924.

17. 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.

18. 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.

19. Balsinde, J., B. Fernández, and J. A. Solís-Herruzo. 1994. Increased incorporation of arachidonic acid into phospholipids in zymosan-stimulated mouse peritoneal macrophages. Eur. J. Biochem. 221: 1013-1018.

20. Balsinde, J., M. A. Balboa, and E. A. Dennis. 2000. Identification of a third pathway for arachidonic acid mobilization and prostaglandin production in activated $P388D_1$ macrophage-like cells. J. Biol. Chem. 275: 22544-22549.

21. Ruipérez, V., A. M. Astudillo, M. A. Balboa, and J. Balsinde. 2009. Coordinate regulation of Toll-like receptor-mediated arachidonic acid mobilization in macrophages by group IVA and group V phospholipase A_2s. *J. Immunol*. 182: 3877–3883.

22. Ruipérez, V., J. Casas, M. A. Balboa, and J. Balsinde. 2007. Group V phospholipase A_2-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages. *J. Immunol.* 179: 631-638.

23. 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 A2/cyclooxygenase-2 pathway. *J. Immunol.* 179: 4821-4828.

24. 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.

25. Pérez, R., M. A. Balboa, and J. Balsinde. 2006. Involvement of group VIA calcium-independent phospholipase A_2 in macrophage engulfment of hydrogen peroxide-treated U937 cells. *J. Immunol.* 176: 2555-2561.