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Review

Dynamics of arachidonic acid mobilization by inflammatory cells

Alma M. Astudillo, David Balgoma, María A. Balboa, Jesús Balsinde *

Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC), 47003 Valladolid, Spain
Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), 08036 Barcelona, Spain

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ABSTRACT

The development of mass spectrometry-based techniques is opening new insights into the understanding of arachidonic acid (AA) metabolism. AA incorporation, remodeling and release are collectively controlled by acyltransferases, phospholipases and transacylases that exquisitely regulate the distribution of AA between the different glycerophospholipid species and its mobilization during cellular stimulation. Traditionally, studies involving phospholipid AA metabolism were conducted by using radioactive precursors and scintillation counting from thin layer chromatography separations that provided only information about lipid classes. Today, the input of lipidomic approaches offers the possibility of characterizing and quantifying specific molecular species with great accuracy and within a biological context associated to protein and/or gene expression in a temporal frame. This review summarizes recent results applying mass spectrometry-based lipidomic approaches to the identification of AA-containing glycerophospholipids, phospholipid AA remodeling and synthesis of oxygenated metabolites.

1. Introduction

The traditional view of lipids typically acting as membrane building blocks or fuel has changed dramatically in recent years. Now, it is widely accepted that these biomolecules are centrally involved in cell signaling, and that imbalances in lipid metabolism are causative agents of a number of high-prevalence disorders, ranging from cardiovascular disease, diabetes and obesity to cancer [1–3]. Hundreds to thousands of lipid species can be identified in a given biological system at a cell or tissue levels, each of them with a particular distribution and function [4].

The significant advances in analytical techniques over the last years, especially mass spectrometry (MS) and the parallel improvement in molecular ionization, together with the possibility to use this technique as a detector in chromatographic separation, have constituted a breakthrough in lipid analysis and, therefore, a better understanding of lipid biochemistry has been possible. This has contributed to the development of lipidomics [5–7], a branch of metabolomics that pursues a thorough scientific study of lipids. Lipidomics not only aims at characterizing and analyzing lipid species in a particular state of the cell — called the *static* composition — but also aims at integrating the understanding of lipid pathways (synthesis, remodeling or transport) with their biological roles and with gene regulation and protein expression [5,7–11].

There are two major strategies for lipidomic analysis. The first one, called global lipidomics, pursues the analysis of the whole lipidome of biological samples, by using either direct infusion methods or previous liquid chromatography steps. On the other hand, targeted or focused lipidomics, is based on setting conditions for analysis of specific categories of lipids, thus improving sensitivity [11–14].

As lipidomics is focused on profiling the *static* composition of molecular species in biological samples, the term metabolipidomics has been coined to emphasize the dynamical aspects of lipids in cells, organs, tissues and whole organisms [15]. In this case, lipids containing stable isotopes are used to follow their metabolic fate through the various possible pathways, thus facilitating the identification of minor species with rapid turnover rates.

2. Cellular utilization of arachidonic acid

Arachidonic acid (5,8,11,14-eicosatetraenoic acid) is an ω-6 essential fatty acid, obtained directly from diet or, alternatively, synthesized from linoleic acid (18:2n−6) through the successive actions of Δ6-desaturase, elongase and Δ5-desaturase, which occurs mainly in liver but also in other tissues (Fig. 1). In western diets it is calculated that the intake of AA is 0.2–0.3 g/day, whereas the intake of linoleic acid is 10–20 g/day, indicating that the amount of AA coming from linoleic acid is higher than that coming directly from the diet [16,17].

AA is the common precursor of the eicosanoids, a family of lipid mediators with key roles in physiology and pathophysiology, particularly

Abbreviations: AA, arachidonic acid; PLA_2, phospholipase A_2; $cPLA_2\alpha$, group IVA cytosolic PLA_2; $iPLA_2$, Ca^{2+}-independent PLA_2; $sPLA_2$, secreted PLA_2; CoA-IT, CoA-independent transacylase; ACS, acyl-CoA synthetase; ESI, electrospray ionization; HPLC, high performance liquid chromatography; MS, mass spectrometry; TLC, thin layer chromatography; PC, choline glycerophospholipids; PE, ethanolamine glycerophospholipids; PI, phosphatidylinositol

* Corresponding author at: University of Valladolid School of Medicine, Calle Sanz y Forés 3, 47003 Valladolid, Spain. Tel.: +34 983 423 062; fax: +34 983 184 800.
E-mail address: jbalsinde@ibgm.uva.es (J. Balsinde).

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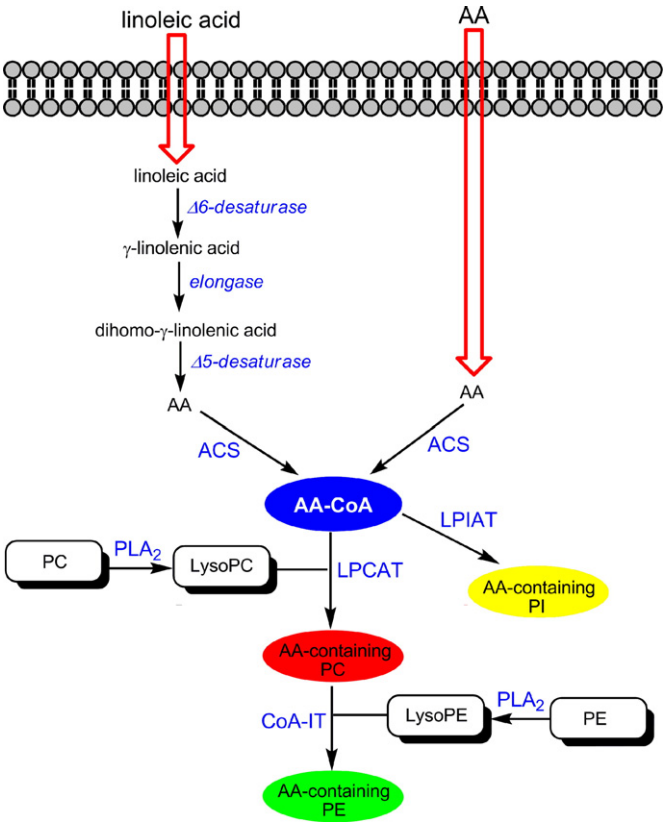

Fig. 1. AA incorporation in glycerophospholipids. AA is obtained directly from diet or synthesized from linoleic acid. Afterward, AA is incorporated into the *sn*-2 position of glycerophospholipids and later subjected to remodeling processes via CoA-IT-dependent reactions. ACS: acyl:CoA synthetase; LPCAT: lysophosphatidylcholine:acyl-CoA acyltransferase; PLA_2: phospholipase A_2; CoA-IT: independent-CoA transacylase.

in inflammatory reactions [18,19], although the fatty acid can exert potent biological roles by itself, e.g. by inducing apoptosis [20–22]. Additionally, when present in elevated concentrations, AA can give rise to significant quantities of its 2-carbon elongation product, adrenic acid (22:4n−6). Adrenic acid is the precursor of 22-carbon 1*a*,1*b*-dihomologue prostaglandins (dihomoprostaglandins) [23–26]. The potent biological activity of the eicosanoids compels the cells to tightly control the levels of free AA in such a manner that availability of free AA is frequently a rate-limiting step in eicosanoid generation [27,28].

In inflammatory cells, AA is generally found esterified in the *sn*-2 position of glycerophospholipids, particularly choline glycerophospholipids (PC), ethanolamine glycerophospholipids (PE), and phosphatidylinositol (PI) [28]. Cellular free AA levels are controlled by two competing reactions; on one hand, the phospholipase A_2 (PLA_2)-mediated cleavage of the *sn*-2 position of phospholipids to yield the free fatty acid and, on the other, the CoA-dependent acyltransferase-modulated acylation reactions that re-incorporate the free fatty acid into phospholipids [29,30]. In resting unstimulated cells, the reacylation reactions dominate over the PLA_2-mediated deacylation step; thus free AA is kept at very low levels. Stimulation of the cells by receptor agonists results in the activation of intracellular PLA_2s [31–35]. Under these conditions, the rate of AA release clearly exceeds that of reincorporation into phospholipids; hence, net accumulation of free AA occurs that is followed by its conversion into various eicosanoids. Nevertheless, AA reacylation reactions are still very significant under stimulated conditions, as manifested by the fact that only a minor fraction of the free AA released by PLA_2s is converted into eicosanoids, the remainder being effectively incorporated back into phospholipids [36,37].

The first step for AA incorporation into cellular lipids is the activation of the carboxyl group of the fatty acid by thioesterification with CoA. This reaction is catalyzed by acyl-CoA synthetases, of which there are many in cells [30]. In the context of AA metabolism it is worth mentioning the long chain acyl-CoA synthetases ACSL3, 4 and 6, as they show some selectivity toward AA [38–40]. The next step in the AA incorporation is the esterification of the fatty acid into primarily the *sn*-2 position of glycerophospholipids, a reaction that is carried out by lysophospholipid:acyl CoA acyltransferases, in particular those of the membrane-bound O-acyltransferase family (MBOAT) [41]. Those that have been found to exhibit clear preference for AA include lysoPC:acyl-CoA acyltransferase 2 [42], lysoPC:acyl-CoA acyltransferase 3 [43–45], lysoPI:acyl-CoA acyltransferase and lysophosphatidic acid:acyl-CoA acyltransferase 3 [44,46,47]. In addition, the lipase CGI-58 shows lysophosphatidic:acyl-CoA acyltransferase with high preference toward AA [48]. For detailed information on the biochemistry and functioning of CoA-dependent acyl transferases, the reader is kindly referred to recent reviews on the subject [30,49–51].

AA bound to phospholipid is also the subject of successive transacylation reactions aimed at ensuring the proper distribution of the fatty acid within the various cellular phospholipid pools [29,30,52–54]. This appears to be important not only for membrane homeostasis but also for the execution of appropriate cell responses during physiological and pathophysiological activation [55–59]. These transacylation reactions are catalyzed by CoA-independent transacylase (CoA-IT), an enzyme that transfers AA moieties preferentially from diacyl PC species to PE plasmalogens [29,30] (Fig. 1). The sequence of CoA-IT is yet to be described. Thus, currently the only manner to study the cell regulation of CoA-IT is by following its enzyme activity [60,61].

Once the AA has been effectively incorporated into phospholipids the fatty acid can be eventually liberated, especially under cell stimulation conditions, by a number of PLA_2 enzymes. So far, more than 30 enzymes possessing PLA_2 activity have been described in mammals [62–64]. Attending to sequence homology criteria, the PLA_2s have been classified into 16 groups [62–65], although based on biochemical features they can be grouped into 5 main families, namely the Ca^{2+}-dependent secreted PLA_2s (sPLA_2), Ca^{2+}-dependent cytosolic PLA_2s (cPLA_2), Ca^{2+}-independent cytosolic PLA_2s (iPLA_2), platelet-activating factor acetylhydrolases, and lysosomal PLA_2s. Of these, the first two have been repeatedly implicated in receptor-mediated AA mobilization in response to a variety of stimuli. Today, it is firmly established that the calcium-dependent cytosolic group IVA $PLA_2\alpha$ (c$PLA_2\alpha$) is the critical enzyme in stimulus-dependent AA mobilization and that, depending on cell type and stimulation conditions, a sPLA_2 may also participate by amplifying the c$PLA_2\alpha$-regulated response [66–91]. Under some conditions, the Ca^{2+}-independent group VIA iPLA_2 may also effect the AA release [92–94] but in other conditions, it mediates phospholipid reacylation reactions by regulating the steady-state level of lysoPC [95–97], reflecting the multiplicity of functions that this enzyme appears to serve depending on cell type [34,98]. For detailed information on the cellular regulation of AA mobilization by PLA_2 enzymes, the reader is kindly referred to the many comprehensive reviews that have been published in recent years covering different aspects of the subject [31–35,51,62–65,99–104].

After the AA has been released from phospholipids under stimulation conditions, it can be metabolized into eicosanoids through four different pathways, namely cyclooxygenase, lipoxygenase, cytochrome-P450 and oxygen species-triggered reactions (Fig. 2). These pathways yield a plethora of compounds, such as prostaglandins, isoprostanes, thromboxane, leukotrienes, lipoxins and epoxyeicosatrienoic acids, all of which act in a paracrine/autocrine manner through specific receptors on the plasma membrane.

For the biosynthesis of prostaglandins and thromboxane from AA, synthesis of PGH_2 via cyclooxygenase is the first step. Cyclooxygenase incorporates molecular O_2 and forms PGG_2 that is subsequently reduced to form PGH_2 by the action of the peroxidase active site. PGH_2 is the substrate of different prostaglandin and thromboxane synthases that are expressed in tissues and cells in a selective-manner and lead to the formation of PGE_2, PGD_2, PGI_2, $PGF_2\alpha$ and TXA_2 [105]. These compounds

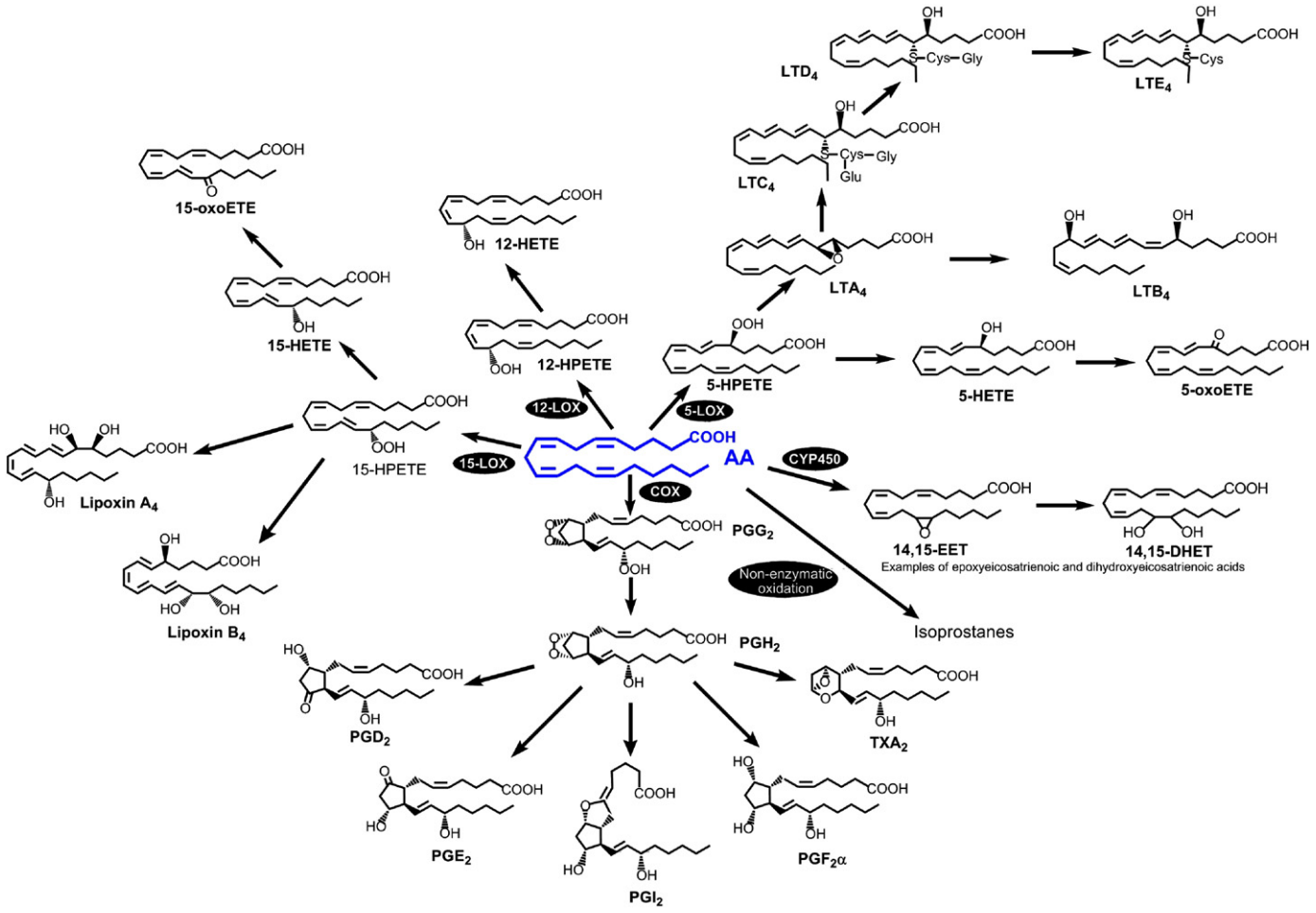

Fig. 2. The four pathways for AA metabolism into eicosanoids are as follows: cyclooxygenase, lipoxygenase, cytochrome-P450 and non-enzymatic oxidation reactions.

exert important biological functions, i.e. modulating smooth muscle tone, vascular permeability, hyperalgesia, fever and platelet aggregation [106].

Leukotrienes, eoxins, lipoxins, and hydroperoxyeicosatetranoic acids (HPETEs) are synthesized from AA by lipoxygenase enzymes (5-, 12- and 15-lipoxygenase) that add molecular O_2 into AA stereospecifically. 5-Lipoxygenase forms 5-hydroperoxyeicosatetraenoic acid (5-HPETE) that is metabolized to LTA_4 in a second step. LTA_4 is unstable, and can rapidly be converted to LTB_4, a potent chemoattractant for polymorphonuclear leukocytes. Additionally, LTA_4 can be converted to LTC_4 which is the precursor of LTD_4 and LTE_4. These three leukotrienes constitute the slow-reacting substances of anaphylaxis that act in the allergic response [107]. Eoxins are produced in a similar manner as the leukotrienes, but by the action of 15-lipoxygenase, and are almost as potent as 5-lipoxygenase-derived leukotrienes [108]. Lipoxins are generated by transcellular biosynthesis and have anti-inflammatory and resolving roles [109].

Other metabolites, including epoxyeicosatrienoic acids (EETs) and their products from epoxy-ring hydrolysis, dihydroxyeicosatrienoic acids (DHETs), are derived from cytochrome-P450 enzymes [110]. EETs have been associated with anti-inflammatory properties in cardiovascular diseases [111].

The fourth pathway of production of eicosanoids is the reaction of AA with reactive oxygen species to yield a group of compounds known as isoprostanes. As radical-driven reactions are not stereoselective, they generate different isomers with structures similar to those of prostaglandins. Because of their origin, isoprostanes have been used as markers of oxidative stress [112].

3. Lipidomic studies of cellular arachidonate metabolism

Traditionally, cellular studies on AA movement to and from glycerophospholipids made extensive use of radiolabeled fatty acid. After lipid extraction and chromatographic separation, quantification was carried out by scintillation counting [54–59,66–76,113–119]. Studies carried out in the 80s and early 90s using this general strategy demonstrated that most of the AA incorporated into cellular phospholipids resides in PC, PE or PI, that the major AA-containing phospholipid classes in many cell types — especially those involved in innate immunity and inflammation reactions — usually contain ether linkages at the *sn*-1 position, and that major remodeling reactions involving AA occur between PC (primarily diacyl) species and PE (primarily alkenyl) species [29,54–56,120–124]. However, a potential problem with the use of radioactive tracers is that sometimes, experiments are not carried out under equilibrium labeling conditions and, therefore, changes detected involve only discrete pools with higher turnover rates. Moreover, using this methodology, it is not possible to ascertain the exact molecular composition of the phospholipids to which AA is bonded.

The advent of HPLC coupled to MS, or direct infusion MS, has made it routinely possible to collect structure information, thus greatly strengthening our knowledge on the cellular utilization of AA. [12–14,125–140]. Moreover, by using a metabolipidomic approach (i.e. combining the power of MS with use of stable isotopes such as deuterium-labeled AA), dynamic information can also be obtained as to the differential mobilization of various cellular AA pools under defined stimulation conditions, thus allowing the understanding of

lipid turnover in contrast with static measures under equilibrium conditions [15,141].

Identification of the phospholipid acyl chains as well as their positioning in the *sn*-1 versus the *sn*-2 position of the glycerol backbone can be obtained through fragmentation experiments (ESI-MS/MS). Such assignments are usually straightforward for analyses of AA-containing PE and PI in the negative ion mode. Analysis of AA-containing PC species is usually carried out in negative mode with postcolumn addition of acetic acid, as $[M+CH_3CO_2]^-$ adducts [14,127,132,136–138,140].

Using $[^2H]_8$AA and reverse phase-HPLC-ESI-MS, Balgoma et al. [142] identified the PI molecular species that initially incorporate AA in human U937 promonocyte-like cells and peripheral blood monocytes. The unusual species PI($[^2H]$AA/$[^2H]$AA) was found to behave as a major but short-lived acceptor of the AA, and its route of biosynthesis was described to involve the direct acylation of both the *sn*-1 and *sn*-2 positions of PI. Likewise, similar studies on the initial incorporation of AA into PE species revealed a fundamental difference between human U937 promonocytes and peripheral blood monocytes in that the former, but not the later, incorporate large quantities into various PE molecular species at short times [61]. This difference is attributed to the higher remodeling rate of U937 cells compared to monocytes, which in turn appears to be related to the intrinsically low AA content that these cells exhibit [61].

Rouzer et al. [143] compared the fatty acid remodeling of murine resident peritoneal macrophages and RAW 264.7 macrophage-like cells after exposure to yeast-derived zymosan. Using tandem MS, AA deficiency of cultured cells compared with their primary counterparts was confirmed, as was the different distribution of AA among phospholipids in AA-enriched RAW 264.7 cells and peritoneal macrophages [143]. These data are relevant in that they emphasize the potential pitfalls of using AA-deficient cells by long term culture in studies of AA metabolism. Although this kind of studies have been useful to describe biochemical pathways, they have not always produced meaningful data from a physiological point of view, i.e. when compared with primary cells, since the AA deficiency of cultured cells usually results in much lower amounts of AA mobilized — and hence of eicosanoids produced — and probably also the preferential use of AA phospholipid pools with higher turnover rates [74,143].

In subsequent studies, Rouzer et al. [144] reported that the bulk of cPLA$_2\alpha$-mediated AA release in zymosan-stimulated macrophages arises from AA-containing PC species whereas AA-containing PE species show little or no changes. Similar to these studies, using reverse phase HPLC-ESI-MS, Balgoma et al. [145] described that all major AA-containing PC and PI species decrease in a cPLA$_2\alpha$-dependent manner in zymosan-stimulated human monocytes, with only minor changes in the levels of AA-containing PE species. Taking into account previous work utilizing $[^3H]$AA-labeled cells under equilibrium labeling conditions [55–58], it seems likely that PE molecules also serve as immediate substrates for stimulus-induced AA mobilization in monocytes/macrophages, but these are rapidly reacylated using AA from other sources, e.g. PC, via CoA-IT-mediated reactions [144,145]. In this regard, a previous lipidomic study with human platelets demonstrated that ethanolamine plasmalogen species constituted by far the major sources of AA mobilized after thrombin stimulation of the cells at very short times, i.e. 90 s [146]. Collectively, these studies highlight a key distinctive advantage of applying an ESI-MS lipidomic approach to the analysis of AA metabolism, that is, the possibility of identifying the specific molecular species of phospholipids that act as donors and acceptors of AA moieties during activation. Thus, conclusions can now be drawn on the molecular specificity of the phospholipid substrate and the phospholipid-hydrolyzing phospholipase(s) involved.

In the above-mentioned studies with human monocytes [145], the levels of the two minor species PI(20:4/20:4) and PC(20:4/20:4) were found to increase in zymosan-activated cells compared with resting cells, and a third species not present in resting cells, PE(16:1/20:4), appeared under stimulation conditions [145]. Analysis of the pathways involved in the synthesis of these three lipids indicated that PI(20:4/20:4) and PC(20:4/20:4) were produced in a deacylation/reacylation pathway via cPLA$_2\alpha$ and acyl-CoA synthetase-dependent reactions, whereas PE(16:1/20:4) was generated via a CoA-independent transacylation reaction [145]. The finding that certain AA-containing species that are detected at low levels or not detected at all under resting conditions, significantly increase in activated cells via selective biosynthetic pathways suggests that they may be regarded as lipid markers of particular activation states of the cells.

Lipidomic profiling of AA-containing phospholipids may also be useful to define cell-specific differences. Fig. 3 compares the distribution of AA-containing phospholipids in human peripheral blood monocytes [145], human monocyte-derived macrophages [147], and mouse peritoneal macrophages [148], all obtained by reverse phase HPLC-ESI-MS under identical experimental conditions. The % total AA in phospholipids versus total phospholipid was 22 ± 1, 20 ± 2, and 36 ± 2 (mean ± S.E.M., $n=3$) in human monocytes, human macrophages, and mouse macrophages respectively. Although the overall distribution of AA between glycerophospholipids is similar in the three cell types, some potentially useful differences are apparent. For example, the plasmalogen species PC(P-16:0/20:4) is present at low levels in human monocytes and mouse macrophages but is by far the major AA-containing PC species of human macrophages. Similarly, PC(16:0/20:4) is present in trace amounts in human monocytes and macrophages but is one of the most abundant AA-containing PC species of mouse macrophages (Fig. 3). As for human monocytes, note the conspicuous absence of the plasmalogen species PE(P-18:1/20:4) and PE(P-18:2/20:4), which is counteracted by the high level of the related species PE(P-18:0/20:4), compared to human and mouse macrophages.

Although not focusing directly on AA-containing phospholipids, other lipidomic studies looking for biomarkers in various macrophage cell types, have led to the identification of unusual glycerophospholipids such as ether-linked PI species, PE species containing very long fatty acyl chains, or phospholipids containing threonine as the polar headgroup [149]. Very recently, combining HPLC-ion trap MS and HPLC-triple quadrupole MS, Yang et al. [150] described changes in glycerophospholipid species in response to different levels of oxidative stress induced by H_2O_2 in EA.hy926 cells. In these studies, the authors identified 7 lysophospholipid correlating with cPLA$_2\alpha$ activation under these conditions, which could potentially be regarded as biomarkers of oxidative stress conditions [150].

More ambitious lipidomic analyses have aimed at establishing the dynamics of lipid metabolism and regulation during inflammatory stimulation. Dennis et al. [151] carried out the first quantitative approach for the characterization of the whole lipidome of an inflammatory cell. Dynamic quantitative MS analyses were carried out, together with transcriptional measurements of genes involved in lipid metabolism in RAW 264.7 macrophage-like cells stimulated with Kdo_2-lipid A, or treated with the pharmacological inhibitor compactin. This work focused on 400 lipid species from all major lipid classes, including fatty acids, acyl-CoAs, eicosanoids, glycerophospholipids, sphingolipids, sterols and glycerolipids [151]. In cells stimulated with the specific TLR4 agonist Kdo_2-lipid A, a good correlation was observed between expression of genes coding for enzymes of lipid metabolism and changes of the corresponding metabolites. However a potential shortcoming is that this information could be misleading if the regulation of enzyme levels is post-transcriptional, as is the case of e.g. cPLA$_2\alpha$ [151]. Increases in cholesterol, lanosterol and desmosterol were also documented, as well as increases in sphingolipid content, consistent with induction of the de novo biosynthesis of sphingolipids. Finally, significant changes in phosphatidic acid and PI species were detected, with increases of saturated and monounsaturated phosphatidic acid species, and decreases of polyunsaturated species such as PI(38:4) — probably PI(18:0/20:4) — at 24 h, raising the possibility that lipid molecules involved in signaling still act beyond 24 h stimulation [151].

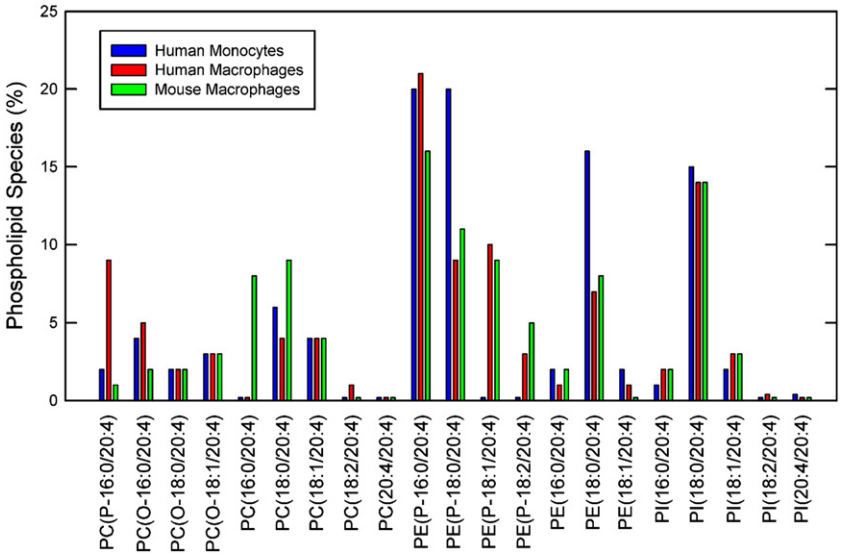

Fig. 3. AA-containing PC, PE and PI species in human monocytes (blue), human monocyte-derived macrophages (red) and mouse peritoneal macrophages (green). Data represent means of three independent determinations (S.E.M.<15% for all determinations), and are given as a percent with respect to the total amount of AA-containing phospholipids. For further details see text. Shorthand notation of glycerophospholipids (abscissa) follows the guidelines proposed by Fahy et al. [155].

The application of MS techniques for eicosanoid quantitation has supposed a significant breakthrough in this field of research, since traditional analyses, based in enzyme-linked immunosorbent assays (ELISA) allow the analysis of only one eicosanoid per assay, thus prohibiting great-scale assays. By using a lipidomic approach, Norris et al. [152] analyzed the eicosanoid profile together with protein and gene expression of enzymes of eicosanoid metabolism when different types of macrophages — murine resident peritoneal macrophages, bone marrow-derived macrophages, thioglycollate elicited macrophages and RAW 264.7 macrophage-like cells — were exposed to Kdo_2-lipid A. Over 140 species were detected, including AA-derived metabolites generated by COX, LOX and CYP450 pathways and those derived from non-enzymatic pathways (Fig. 2). Lower levels of ω-3 fatty acid-derived protectins and resolvins were also detected. In addition, adrenic acid-derived dihomoprostaglandins [23–25] were detected in all macrophage types, suggesting that these compounds may possess biological significance. Overall, the data confirmed that although there are potentially many factors that affect eicosanoid production such PLA_2 activation, the enzymes of the AA reacylation pathway (Fig. 1), and terminal synthases, the delayed phase of prostaglandin production by all macrophage types was strikingly dependent on the maximal level of COX-2 expression [152].

Andreyev et al. [153] focused their lipidomic analyses on the different subcellular localization of lipid species in RAW 264.7 cells, analyzing a total of 229 species in various compartments such as endoplasmic reticulum, mitochondria, nuclei, plasmalemma and cytoplasm, and addressing the changes occurring upon activation with Kdo_2-lipid A. Cell activation led to remodeling of the lipidome in all subcellular compartments, apparently in an organelle-specific manner. Changes detected included increases in phosphatidic acid in the endoplasmic reticulum — suggestive of phospholipase D activation in that compartment — or increases in highly unsaturated cardiolipins and oxidized sterols in mitochondria — suggestive of oxidative stress in this organelle. In addition, ether-linked phospholipids increased in plasma membrane but decreased in the endoplasmic reticulum under stimulation conditions, especially the ether species PE(P-38:4/O-38:5), which probably contains AA [153].

4. Concluding remarks and future perspectives

The robustness and sensitivity of mass spectrometry converts this technique in the preferred tool for lipidomic analyses, which focuses on the complete characterization of lipid species in combination with gene/protein expression under different conditions or aiming at the understanding of turnover and lipid pathways. In this regard, lipidomic approaches, specifically the use of ESI-MS/MS with or without a previous HPLC separation and alternative use of deuterated compounds, permit the thorough characterization of cellular AA dynamics with regard to incorporation into and remodeling between different phospholipid classes, and the liberation of the fatty acid and oygenation to form eicosanoids. Current analysis and characterization of these processes in the context of lipidomics have helped to understand new regulatory features of AA pathways of physiological and pathophysiological relevance. The study of AA regulation along with data from proteomics and genomics will enable the in-depth knowledge of the role of AA in different cell types and patients. This holistic point of view will put AA trafficking and metabolism in the context of systems biology approaches [154].

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References

[1] T. Yeung, S. Grinstein, Lipid signaling and the modulation of surface charge during phagocytosis, Immunol. Rev. 219 (2007) 17–36.
[2] M.P. Wymann, R. Schneiter, Lipid signalling in disease, Nat. Rev. Mol. Cell Biol. 9 (2008) 162–176.
[3] M. Bou Khalil, W. Hou, H. Zhou, F. Elisma, L.A. Swayne, A.P. Blanchard, Z. Yao, S.A. Bennett, D. Figeys, Lipidomics era: accomplishments and challenges, Mass Spectrom. Rev. 29 (2010) 877–929.
[4] A.M. Hicks, C.J. DeLong, M.J. Thomas, M. Samuel, Z. Cui, Unique molecular signatures of glycerophospholipid species in different rat tissues analyzed by tandem mass spectrometry, Biochim. Biophys. Acta 1761 (2006) 1022–1029.
[5] F. Spener, M. Lagarde, A. Geloen, M. Record, What is lipidomics? Eur. J. Lipid Sci. Technol. 105 (2003) 481–482.
[6] A.D. Watson, Lipidomics: a global approach to lipid analysis in biological systems, J. Lipid Res. 47 (2006) 2101–2111.
[7] M.R. Wenk, Lipidomics: new tools and applications, Cell 143 (2010) 888–895.
[8] J.B. German, L.A. Gillies, J.T. Smilowitz, A.M. Zivkovic, S.M. Watkins, Lipidomics and lipid profiling in metabolomics, Curr. Opin. Lipidol. 18 (2007) 66–71.
[9] E.A. Dennis, Lipidomics joins the omics evolution, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 2089–2090.
[10] G. van Meer, Cellular lipidomics, EMBO J. 24 (2005) 3159–3165.
[11] M.J. Wakelam, T.R. Pettitt, A.D. Postle, Lipidomic analysis of signaling pathways, Methods Enzymol. 432 (2007) 233–246.

[12] X. Han, R.W. Gross, Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics, J. Lipid Res. 44 (2003) 1071–1079.
[13] R. Taguchi, T. Houjou, H. Nakanishi, T. Yamazaki, M. Ishida, M. Imagawa, T. Shimizu, Focused lipidomics by tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 823 (2005) 26–36.
[14] X. Han, K. Yang, R.W. Gross, Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses, Mass Spectrom. Rev. 31 (2012) 134–178.
[15] O.B. Bleijerveld, M. Houweling, M.J. Thomas, Z. Cui, Metabolipidomics: profiling metabolism of glycerophospholipid species by stable isotopic precursors and tandem mass spectrometry, Anal. Biochem. 352 (2006) 1–14.
[16] H. Sprecher, Metabolism of highly unsaturated n−3 and n−6 fatty acids, Biochim. Biophys. Acta 1486 (2000) 219–231.
[17] L. Zhou, A. Nilsson, Sources of eicosanoid precursor fatty acid pools in tissues, J. Lipid Res. 42 (2001) 1521–1542.
[18] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.
[19] J.W. Phillis, L.A. Horrocks, A.A. Farooqui, Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders, Brain Res. Rev. 52 (2006) 201–243.
[20] Y. Cao, A.T. Pearman, G.A. Zimmerman, T.M. McIntyre, S.M. Prescott, Intracellular unesterified arachidonic acid signals apoptosis, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 11280–11285.
[21] S. Serini, E. Piccioni, N. Merendino, G. Calviello, Dietary polyunsaturated fatty acids as inducers of apoptosis: implications for cancer, Apoptosis 14 (2009) 135–152.
[22] J. Balsinde, R. Pérez, M.A. Balboa, Calcium-independent phospholipase A_2 and apoptosis, Biochim. Biophys. Acta 1761 (2006) 1344–1350.
[23] R. Harkewicz, E. Fahy, A. Andreyev, E.A. Dennis, Arachidonate-derived dihomo-prostaglandin production observed in endotoxin-stimulated macrophage-like cells, J. Biol. Chem. 282 (2007) 2899–2910.
[24] H. Sprecher, M. VanRollins, F. Sun, A. Wyche, P. Needleman, Dihomo-prostaglandins and -thromboxane. A prostaglandin family from adrenic acid that may be preferentially synthesized in the kidney, J. Biol. Chem. 257 (1982) 3912–3918.
[25] M. VanRollins, L. Horrocks, H. Sprecher, Metabolism of 7,10,13,16-docosatetraenoic acid to dihomo-thromboxane, 14-hydroxy-7,10,12-nonadecatrienoic acid and hydroxy fatty acids by human platelets, Biochim. Biophys. Acta 833 (1985) 272–280.
[26] P.G. Kopf, D.X. Zhang, K.M. Gauthier, K. Nithipatikom, X.Y. Yi, J.R. Falck, W.B. Campbell, Adrenic acid metabolites as endogenous endothelium-derived and zona glomerulosa-derived hyperpolarizing factors, Hypertension 55 (2010) 547–554.
[27] I. Flesch, T. Schonhardt, E. Ferber, Phospholipases and acyltransferases in macrophages, Klin. Wochenschr. 67 (1989) 119–122.
[28] R.F. Irvine, How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J. 204 (1982) 3–16.
[29] F.H. Chilton, A.N. Fonteh, M.E. Surette, M. Triggiani, J.D. Winkler, Control of arachidonate levels within inflammatory cells, Biochim. Biophys. Acta 1299 (1996) 1–15.
[30] G. Pérez-Chacón, A.M. Astudillo, D. Balgoma, M.A. Balboa, J. Balsinde, Control of free arachidonic acid levels by phospholipases A_2 and lysophospholipid acyltransferases, Biochim. Biophys. Acta 1791 (2009) 1103–1113.
[31] J. Balsinde, M.V. Winstead, E.A. Dennis, Phospholipase A_2 regulation of arachidonic acid mobilization, FEBS Lett. 531 (2002) 2–6.
[32] J. Balsinde, M.A. Balboa, P.A. Insel, E.A. Dennis, Regulation and inhibition of phospholipase A_2, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 175–189.
[33] C.C. Leslie, Regulation of arachidonic acid availability for eicosanoid production, Biochem. Cell Biol. 82 (2004) 1–17.
[34] J. Balsinde, M.A. Balboa, Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A_2 in activated cells, Cell. Signal. 17 (2005) 1052–1062.
[35] M.A. Balboa, J. Balsinde, Oxidative stress and arachidonic acid mobilization, Biochim. Biophys. Acta 1761 (2006) 385–391.
[36] J. Balsinde, B. Fernández, J.A. Solís-Herruzo, Increased incorporation of arachidonic acid into phospholipids in zymosan-stimulated mouse peritoneal macrophages, Eur. J. Biochem. 221 (1994) 1013–1018.
[37] G. Pérez-Chacón, A.M. Astudillo, V. Ruipérez, M.A. Balboa, J. Balsinde, Signaling role for lysophosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes, J. Immunol. 184 (2010) 1071–1078.
[38] T. Fujino, M.J. Kang, H. Suzuki, H. Iijima, T. Yamamoto, Molecular characterization and expression of rat acyl-CoA synthetase 3, J. Biol. Chem. 271 (1996) 16748–16752.
[39] M.J. Kang, T. Fujino, H. Sasano, H. Minekura, N. Yabuki, H. Nagura, H. Iijima, T.T. Yamamoto, A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 2880–2884.
[40] Y. Cao, E. Traer, G.A. Zimmerman, T.M. McIntyre, S.M. Prescott, Cloning, expression, and chromosomal localization of human long-chain fatty acid-CoA ligase 4 (FACL4), Genomics 49 (1998) 327–330.
[41] H. Shindou, M. Eto, R. Morimoto, T. Shimizu, Identification of membrane O-acyltransferase family motifs, Biochem. Biophys. Res. Commun. 383 (2009) 320–325.
[42] H. Shindou, D. Hishikawa, H. Nakanishi, T. Harayama, S. Ishii, R. Taguchi, T. Shimizu, A single enzyme catalyzes both platelet-activating factor production and membrane biogenesis of inflammatory cells. Cloning and characterization of acetyl-CoA:Lyso-PAF acetyltransferase, J. Biol. Chem. 282 (2007) 6532–6539.
[43] D. Hishikawa, H. Shindou, S. Kobayashi, H. Nakanishi, R. Taguchi, T. Shimizu, Discovery essential of a lysophospholipid acyltransferase family for membrane asymmetry and diversity, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2830–2835.
[44] M.A. Gijón, W.R. Riekhof, S. Zarini, R.C. Murphy, D.R. Voelker, Lysophospholipid acyltransferases and arachidonate recycling in human neutrophils, J. Biol. Chem. 283 (2008) 30235–30245.
[45] Y. Zhao, Y.Q. Chen, T.M. Bonacci, D.S. Bredt, S.Y. Li, W.R. Bensch, D.E. Moller, M. Kowala, R.J. Konrad, G.Q. Cao, Identification and characterization of a major liver lysophosphatidylcholine acyltransferase, J. Biol. Chem. 283 (2008) 8258–8265.
[46] K. Yuki, H. Shindou, D. Hishikawa, T. Shimizu, Characterization of mouse lysophosphatidic acid acyltransferase 3: an enzyme with dual functions in the testis, J. Lipid Res. 50 (2009) 860–869.
[47] H.C. Lee, T. Inoue, R. Imae, N. Kono, S. Shirae, S. Matsuda, K. Gengyo-Ando, S. Mitani, H. Arai, *Caenorhabditis elegans* mboa-7, a member of the MBOAT family, is required for selective incorporation of polyunsaturated fatty acids into phosphatidylinositol, Mol. Biol. Cell 19 (2008) 1174–1184.
[48] G. Montero-Morán, J.M. Caviglia, D. McMahon, A. Rothenberg, V. Subramanian, Z. Xu, S. Lara-González, J. Storch, G.M. Carman, D.L. Brasaemle, CGI-58/ABHD5 is a coenzyme A-dependent lysophosphatidic acid acyltransferase, J. Lipid Res. 51 (2010) 709–719.
[49] H. Shindou, D. Hishikawa, T. Harayama, K. Yuki, T. Shimizu, Recent progress on acyl CoA: lysophospholipid acyltransferase research, J. Lipid Res. 50 (2009) S46–S51.
[50] H. Shindou, T. Shimizu, Acyl-CoA:lysophospholipid acyltransferases, J. Biol. Chem. 284 (2009) 1–5.
[51] T. Shimizu, Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation, Annu. Rev. Pharmacol. Toxicol. 49 (2009) 123–150.
[52] J.I. MacDonald, H. Sprecher, Phospholipid fatty acid remodeling in mammalian cells, Biochim. Biophys. Acta 1084 (1991) 105–121.
[53] M. Hermansson, K. Hokynar, P. Somerharju, Mechanisms of glycerophospholipid homeostasis in mammalian cells, Prog. Lipid Res. 50 (2011) 240–257.
[54] F.H. Chilton, R.C. Murphy, Remodeling of arachidonate-containing phosphoglycerides within the human neutrophil, J. Biol. Chem. 261 (1986) 7771–7777.
[55] M.L. Nieto, M.E. Venable, S.A. Bauldry, D.G. Greene, M. Kennedy, D.A. Bass, R.L. Wykle, Evidence that hydrolysis of ethanolamine plasmalogens triggers synthesis of platelet-activating factor via a transacylation reaction, J. Biol. Chem. 266 (1991) 18699–18706.
[56] A.N. Fonteh, F.H. Chilton, Rapid remodeling of arachidonate from phosphatidylcholine to phosphatidylethanolamine pools during mast cell activation, J. Immunol. 148 (1992) 1784–1791.
[57] J. Balsinde, E.A. Dennis, Distinct roles in signal transduction for each of the phospholipase A_2 enzymes present in $P388D_1$ macrophages, J. Biol. Chem. 271 (1996) 6758–6765.
[58] E. Boilard, M.E. Surette, Anti-CD3 and concanavalin A-induced human T cell proliferation is associated with an increased rate of arachidonate-phospholipid remodeling, J. Biol. Chem. 276 (2001) 17568–17575.
[59] J. Balsinde, Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling, Biochem. J. 364 (2002) 695–702.
[60] J.D. Winkler, C.M. Sung, C.F. Bennett, F.H. Chilton, Characterization of CoA-independent transacylase activity in U937 cells, Biochim. Biophys. Acta 1081 (1991) 339–346.
[61] A.M. Astudillo, G. Pérez-Chacón, D. Balgoma, L. Gil-de-Gómez, V. Ruipérez, C. Guijas, M.A. Balboa, J. Balsinde, Influence of cellular arachidonic acid levels on phospholipid remodeling and CoA-independent transacylase activity in human monocytes and U937 cells, Biochim. Biophys. Acta 1811 (2011) 97–103.
[62] J.E. Burke, E.A. Dennis, Phospholipase A_2 structure/function, mechanism, and signaling, J. Lipid Res. 50 (2009) S237–S242.
[63] R.H. Schaloske, E.A. Dennis, The phospholipase A_2 superfamily and its group numbering system, Biochim. Biophys. Acta 1761 (2006) 1246–1259.
[64] D.A. Six, E.A. Dennis, The expanding superfamily of phospholipase A_2 enzymes: classification and characterization, Biochim. Biophys. Acta 1488 (2000) 1–19.
[65] M. Murakami, Y. Taketomi, Y. Miki, H. Sato, T. Hirabayashi, K. Yamamoto, Recent progress in phospholipase A_2 research: from cells to animals to humans, Prog. Lipid Res. 50 (2011) 152–192.
[66] Z.H. Qiu, M.A. Gijón, M.S. de Carvalho, D.M. Spencer, C.C. Leslie, The role of calcium and phosphorylation of cytosolic phospholipase A_2 in regulating arachidonic acid release in macrophages, J. Biol. Chem. 273 (1998) 8203–8211.
[67] H. Fujishima, R.O. Sanchez Mejia, C.O. Bingham, B.K. Lam, A. Sapirstein, J.V. Bonventre, K.F. Austen, J.P. Arm, Cytosolic phospholipase A_2 is essential for both the immediate and the delayed phases of eicosanoid generation in mouse bone marrow-derived mast cells, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4803–4807.
[68] J. Balsinde, M.A. Balboa, P.A. Insel, E.A. Dennis, Differential regulation of phospholipase D and phospholipase A_2 by protein kinase C in $P388D_1$ macrophages, Biochem. J. 321 (1997) 805–809.
[69] M. Murakami, T. Kambe, S. Shimbara, K. Higashino, K. Hanasaki, H. Arita, M. Horiguchi, M. Arita, H. Arai, K. Inoue, I. Kudo, Different functional aspects of the group II subfamily (types IIA and V) and type X secretory phospholipase A_2s in regulating arachidonic acid release and prostaglandin generation, J. Biol. Chem. 274 (1999) 31435–31444.
[70] H. Shinohara, M.A. Balboa, C.A. Johnson, J. Balsinde, E.A. Dennis, Regulation of delayed prostaglandin production in activated $P388D_1$ macrophages by group IV cytosolic and group V secretory phospholipase A_2s, J. Biol. Chem. 274 (1999) 12263–12268.

[71] J. Balsinde, M.A. Balboa, S. Yedgar, E.A. Dennis, Group V phospholipase A_2-mediated oleic acid mobilization in lipopolysaccharide-stimulated $P388D_1$ macrophages, J. Biol. Chem. 275 (2000) 4783–4786.

[72] J. Marshall, E. Krump, T. Lindsay, G. Downey, D.A. Ford, P. Zhu, P. Walker, B. Rubin, Involvement of cytosolic phospholipase A_2 and secretory phospholipase A_2 in arachidonic acid release from human neutrophils, J. Immunol. 164 (2000) 2084–2091.

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

[74] M.A. Gijón, D.M. Spencer, A.R. Siddiqi, J.V. Bonventre, C.C. Leslie, Cytosolic phospholipase A_2 is required for macrophage arachidonic acid release by agonists that do and do not mobilize calcium. Novel role of mitogen-activated protein kinase pathways in cytosolic phospholipase A_2 regulation, J. Biol. Chem. 275 (2000) 20146–20156.

[75] M. Murakami, R.S. Koduri, A. Enomoto, S. Shimbara, M. Seki, K. Yoshihara, A. Singer, E. Valentin, F. Ghomashchi, G. Lambeau, M.H. Gelb, I. Kudo, Distinct arachidonate-releasing functions of mammalian secreted phospholipase A_2s in human embryonic kidney 293 and rat mastocytoma RBL-2H3 cells through heparan sulfate shuttling and external plasma membrane mechanisms, J. Biol. Chem. 276 (2001) 10083–10096.

[76] M.A. Balboa, R. Pérez, J. Balsinde, Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A_2 is regulated by cytosolic phospholipase A_2-derived hydroperoxyeicosatetraenoic acid, J. Immunol. 171 (2003) 989–994.

[77] M.A. Balboa, Y. Shirai, G. Gaietta, M.H. Ellisman, J. Balsinde, E.A. Dennis, Localization of group V phospholipase A_2 in caveolin-enriched granules in activated $P388D_1$ macrophage-like cells, J. Biol. Chem. 278 (2003) 48059–48065.

[78] Y. Satake, B.L. Díaz, B. Balestrieri, B.K. Lam, Y. Kanaoka, M.J. Grusby, J.P. Arm, Role of group V phospholipase A_2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption, J. Biol. Chem. 279 (2004) 16488–16494.

[79] C. Mounier, F. Ghomashchi, M.R. Lindsay, S. James, A.G. Singer, R.G. Parton, M.H. Gelb, Arachidonic acid release from mammalian cells transfected with human groups IIA and X secreted phospholipase A_2 predominantly during the secretory process and with the involvement of cytosolic phospholipase $A_2\alpha$, J. Biol. Chem. 279 (2004) 25024–25038.

[80] Y. Shirai, J. Balsinde, E.A. Dennis, Localization and functional interrelationships among cytosolic group IV, secreted group V, and Ca^{2+}-independent group VI phospholipase A_2s in $P388D_1$ macrophages using GFP/RFP constructs, Biochim. Biophys. Acta 1735 (2005) 119–129.

[81] H. Kuwata, T. Nonaka, M. Murakami, I. Kudo, Search of factors that intermediate cytokine-induced group IIA phospholipase A_2 expression through the cytosolic phospholipase A_2-and 12/15-lipoxygenase-dependent pathway, J. Biol. Chem. 280 (2005) 25830–25839.

[82] J. Casas, M.A. Gijón, A.G. Vigo, M.S. Crespo, J. Balsinde, M.A. Balboa, Phosphatidylinositol 4,5-bisphosphate anchors cytosolic group IVA phospholipase A_2 to perinuclear membranes and decreases its calcium requirement for translocation in live cells, Mol. Biol. Cell 17 (2006) 155–162.

[83] G.T. Wijewickrama, J.H. Kim, Y.J. Kim, A. Abraham, Y. Oh, B. Ananthanarayanan, M. Kwatia, S.J. Ackerman, W. Cho, Systematic evaluation of transcellular activities of secretory phospholipases A_2. High activity of group V phospholipase A_2 to induce eicosanoid biosynthesis in neighboring inflammatory cells, J. Biol. Chem. 281 (2006) 10935–10944.

[84] B.L. Diaz, Y. Satake, E. Kikawada, B. Balestrieri, J.P. Arm, Group V secretory phospholipase A_2 amplifies the induction of cyclooxygenase 2 and delayed prostaglandin D_2 generation in mouse bone marrow culture-derived mast cells in a strain-dependent manner, Biochim. Biophys. Acta 1761 (2006) 1489–1497.

[85] J. Pindado, J. Balsinde, M.A. Balboa, TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A_2/cyclooxygenase-2 pathway, J. Immunol. 179 (2007) 4821–4828.

[86] E. Kikawada, J.V. Bonventre, J.P. Arm, Group V secretory PLA2 regulates TLR2-dependent eicosanoid generation in mouse mast cells through amplification of ERK and $cPLA_2\alpha$ activation, Blood 110 (2007) 561–567.

[87] V. Ruipérez, J. Casas, M.A. Balboa, J. Balsinde, Group V phospholipase A_2-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages, J. Immunol. 179 (2007) 631–638.

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

[89] S. Suram, T.A. Gangelhoff, P.R. Taylor, M. Rosas, G.D. Brown, J.V. Bonventre, S. Akira, S. Uematsu, D.L. Williams, R.C. Murphy, C.C. Leslie, Pathways regulating cytosolic phospholipase A_2 activation and eicosanoid production in macrophages by *Candida albicans*, J. Biol. Chem. 285 (2010) 30676–30685.

[90] K.J. Bryant, M.J. Bidgood, P.W. Lei, M. Taberner, C. Salom, V. Kumar, L. Lee, W.B. Church, B. Courtenay, B.P. Smart, M.H. Gelb, M.A. Cahill, G.G. Graham, H.P. McNeil, K.F. Scott, A bifunctional role for group IIA secreted phospholipase A_2 in human rheumatoid fibroblast-like synoviocyte arachidonic acid metabolism, J. Biol. Chem. 286 (2011) 2492–2503.

[91] W.K. Han, A. Sapirstein, C.C. Hung, A. Alessandrini, J.V. Bonventre, Cross-talk between cytosolic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$) and secretory phospholipase A_2 ($sPLA_2$) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: $sPLA_2$ regulates $cPLA_{2\alpha}$ activity that is responsible for arachidonic acid release, J. Biol. Chem. 278 (2003) 24153–24163.

[92] D.M. Nikolic, M.C. Gong, J. Turk, S.R. Post, Class A scavenger receptor-mediated macrophage adhesion requires coupling of calcium-independent phospholipase A_2 and 12/15-lipoxygenase to Rac and Cdc42 activation, J. Biol. Chem. 282 (2007) 33405–33411.

[93] M.A. Balboa, J. Balsinde, Involvement of calcium-independent phospholipase A_2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells, J. Biol. Chem. 277 (2002) 40384–40389.

[94] C.R. Yellaturu, G.N. Rao, A requirement for calcium-independent phospholipase A_2 in thrombin-induced arachidonic acid release and growth in vascular smooth muscle cells, J. Biol. Chem. 278 (2003) 43831–43837.

[95] J. Balsinde, M.A. Balboa, E.A. Dennis, Antisense inhibition of group VI Ca^{2+}-independent phospholipase A_2 blocks phospholipid fatty acid remodeling in murine $P388D_1$ macrophages, J. Biol. Chem. 272 (1997) 29317–29321.

[96] R. Pérez, R. Melero, M.A. Balboa, J. Balsinde, Role of group VIA calcium-independent phospholipase A_2 in arachidonic acid release, phospholipid fatty acid incorporation, and apoptosis in U937 cells responding to hydrogen peroxide, J. Biol. Chem. 279 (2004) 40385–40391.

[97] R. Pérez, X. Matabosch, A. Llebaria, M.A. Balboa, J. Balsinde, Blockade of arachidonic acid incorporation into phospholipids induces apoptosis in U937 promonocytic cells, J. Lipid Res. 47 (2006) 484–491.

[98] M.V. Winstead, J. Balsinde, E.A. Dennis, Calcium-independent phospholipase A_2: structure and function, Biochim. Biophys. Acta 1488 (2000) 28–39.

[99] M. Ghosh, D.E. Tucker, S.A. Burchett, C.C. Leslie, Properties of the group IV phospholipase A_2 family, Prog. Lipid Res. 45 (2006) 487–510.

[100] G. Lambeau, M.H. Gelb, Biochemistry and physiology of mammalian secreted phospholipases A_2, Annu. Rev. Biochem. 77 (2008) 495–520.

[101] C.C. Leslie, Regulation of the specific release of arachidonic acid by cytosolic phospholipase A_2, Prostaglandins Leukot. Essent. Fatty Acids 70 (2004) 373–376.

[102] T. Hirabayashi, T. Murayama, T. Shimizu, Regulatory mechanism and physiological role of cytosolic phospholipase A_2, Biol. Pharm. Bull. 27 (2004) 1168–1173.

[103] J.V. Bonventre, A. Sapirstein, Group IV cytosolic phospholipase A_2 function: insights from the knockout mouse, Adv. Exp. Med. Biol. 507 (2002) 25–31.

[104] E.A. Dennis, J. Cao, Y.H. Hsu, V. Magriotti, G. Kokotos, Phospholipase A_2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention, Chem. Rev. 111 (2011) 6130–6185.

[105] M.W. Buczynski, D.S. Dumlao, E.A. Dennis, An integrated omics analysis of eicosanoid biology, J. Lipid Res. 50 (2009) 1015–1038.

[106] M.J. Stables, D.W. Gilroy, Old and new generation lipid mediators in acute inflammation and resolution, Prog. Lipid Res. 50 (2011) 35–51.

[107] B. Samuelsson, S.E. Dahlen, J.A. Lindgren, C.A. Rouzer, C.N. Serhan, Leukotrienes and lipoxins — structures, biosynthesis, and biological effects, Science 237 (1987) 1171–1176.

[108] S. Feltenmark, N. Gautam, A. Brunnström, W. Griffiths, L. Backman, C. Edenius, L. Lindbom, M. Björkholm, H.E. Claesson, Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 680–685.

[109] G. Fredman, C.N. Serhan, Specialized proresolving mediator targets for RvE1 and RvD1 in peripheral blood and mechanisms of resolution, Biochem. J. 437 (2011) 185–197.

[110] D. Sacerdoti, A. Gatta, J.C. McGiff, Role of cytochrome P450-dependent arachidonic acid metabolites in liver physiology and pathophysiology, Prostaglandins Other Lipid Mediat. 72 (2003) 51–71.

[111] A.A. Spector, X. Fang, G.D. Snyder, N.L. Weintraub, Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function, Prog. Lipid Res. 43 (2004) 55–90.

[112] U. Jahn, J.M. Galano, T. Durand, Beyond prostaglandins — chemistry and biology of cyclic oxygenated metabolites formed by free radical pathways from polyunsaturated fatty acids, Angew. Chem. Int. Ed. 47 (2008) 5894–5955.

[113] E. Diez, J. Balsinde, M. Aracil, A. Schüller, Ethanol induces release of arachidonic acid but not synthesis of eicosanoids in mouse peritoneal macrophages, Biochim. Biophys. Acta 921 (1987) 82–89.

[114] J. Balsinde, B. Fernández, J.A. Solís-Herruzo, Pathways for arachidonic acid mobilization in zymosan-stimulated mouse peritoneal macrophages, Biochim. Biophys. Acta 1136 (1992) 75–82.

[115] J. Balsinde, B. Fernández, E. Diez, Regulation of arachidonic acid release in mouse peritoneal macrophages. The role of extracellular calcium and protein kinase C, J. Immunol. 144 (1990) 4298–4304.

[116] J. Balsinde, S.E. Barbour, I.D. Bianco, E.A. Dennis, Arachidonic acid mobilization in $P388D_1$ macrophages is controlled by two distinct Ca^{2+}-dependent phospholipase A_2 enzymes, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 11060–11064.

[117] J. Balsinde, M.A. Balboa, E.A. Dennis, Inflammatory activation of arachidonic acid signaling in murine $P388D_1$ macrophages via sphingomyelin synthesis, J. Biol. Chem. 272 (1997) 20373–20377.

[118] J. Balsinde, M.A. Balboa, E.A. Dennis, Functional coupling between secretory phospholipase A_2 and cyclooxygenase-2 and its regulation by cytosolic group IV phospholipase A_2, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 7951–7956.

[119] M.A. Balboa, Y. Sáez, J. Balsinde, Calcium-independent phospholipase A_2 is required for lysozyme secretion in U937 promonocytes, J. Immunol. 170 (2003) 5276–5280.

[120] F.H. Chilton, R.C. Murphy, Stimulated production and natural occurrence of 1,2-diarachidonoylglycerophosphocholine in human neutrophils, Biochem. Biophys. Res. Commun. 145 (1987) 1126–1133.

[121] F.H. Chilton, J.S. Hadley, R.C. Murphy, Incorporation of arachidonic acid into 1-acyl-2-lyso-sn-glycero-3-phosphocholine of the human neutrophil, Biochim. Biophys. Acta 917 (1987) 48–56.

[122] F.H. Chilton, T.R. Connell, 1-Ether-linked phosphoglycerides, major endogenous sources of arachidonate in the human neutrophil, J. Biol. Chem. 263 (1988) 5260–5265.

[123] H.W. Mueller, J.T. O'Flaherty, D.G. Greene, M.P. Samuel, R.L. Wykle, 1-O-alkyl-linked glycerophospholipids of human neutrophils: distribution of arachidonate and other acyl residues in the ether-linked and diacyl species, J. Lipid Res. 25 (1984) 383–388.
[124] T. Sugiura, O. Katayama, J. Fukui, Y. Nakagawa, K. Waku, Mobilization of arachidonic acid between diacyl and ether phospholipids in rabbit alveolar macrophages, FEBS Lett. 165 (1984) 273–276.
[125] R.C. Murphy, J. Fiedler, J. Hevko, Analysis of nonvolatile lipids by mass spectrometry, Chem. Rev. 101 (2001) 479–526.
[126] M. Pulfer, R.C. Murphy, Electrospray mass spectrometry of phospholipids, Mass Spectrom. Rev. 22 (2003) 332–364.
[127] X. Han, R.W. Gross, Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples, Mass Spectrom. Rev. 24 (2005) 367–412.
[128] L.D. Roberts, G. McCombie, C.M. Titman, J.L. Griffin, A matter of fat: an introduction to lipidomic profiling methods, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 871 (2008) 174–181.
[129] T.M. Annesley, Ion suppression in mass spectrometry, Clin. Chem. 49 (2003) 1041–1044.
[130] M. Stahlman, C.S. Ejsing, K. Tarasov, J. Perman, J. Boren, K. Ekroos, High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2664–2672.
[131] C.S. Ejsing, T. Moehring, U. Bahr, E. Duchoslav, M. Karas, K. Simons, A. Shevchenko, Collision-induced dissociation pathways of yeast sphingolipids and their molecular profiling in total lipid extracts: a study by quadrupole TOF and linear ion trap-orbitrap mass spectrometry, J. Mass Spectrom. 41 (2006) 372–389.
[132] A. Larsen, S. Uran, P.B. Jacobsen, T. Skotland, Collision-induced dissociation of glycero phospholipids using electrospray ion-trap mass spectrometry, Rapid Commun. Mass Spectrom. 15 (2001) 2393–2398.
[133] K. Ekroos, C.S. Ejsing, U. Bahr, M. Karas, K. Simons, A. Shevchenko, Charting molecular composition of phosphatidylcholines by fatty acid scanning and ion trap MS3 fragmentation, J. Lipid Res. 44 (2003) 2181–2192.
[134] Z. Cui, M.J. Thomas, Phospholipid profiling by tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2709–2715.
[135] C.S. Ejsing, E. Duchoslav, J. Sampaio, K. Simons, R. Bonner, C. Thiele, K. Ekroos, A. Shevchenko, Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning, Anal. Chem. 78 (2006) 6202–6214.
[136] D. Balgoma, O. Montero, M.A. Balboa, J. Balsinde, Lipidomic approaches to the study of phospholipase A_2-regulated phospholipid fatty acid incorporation and remodeling, Biochimie 92 (2010) 645–650.
[137] E. Vernooij, J. Brouwers, J.J. Kettenes-van den Bosch, D.J.A. Crommelin, RP-HPLC/ESI MS determination of acyl chain positions in phospholipids, J. Sep. Sci. 25 (2002) 285–289.
[138] F.F. Hsu, J. Turk, Electrospray ionization with low-energy collisionally activated dissociation tandem mass spectrometry of glycerophospholipids: mechanisms of fragmentation and structural characterization, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2673–2695.
[139] R. Harkewicz, E.A. Dennis, Applications of mass spectrometry to lipids and membranes, Annu. Rev. Biochem. 80 (2011) 301–325.
[140] R.C. Murphy, P.H. Axelsen, Mass spectrometric analysis of long-chain lipids, Mass Spectrom. Rev. 30 (2011) 579–599.
[141] A.D. Postle, A.N. Hunt, Dynamic lipidomics with stable isotope labelling, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2716–2721.
[142] D. Balgoma, O. Montero, M.A. Balboa, J. Balsinde, Calcium-independent phospholipase A_2-mediated formation of 1,2-diarachidonoyl-glycerophosphoinositol in monocytes, FEBS J. 275 (2008) 6180–6191.
[143] C.A. Rouzer, P.T. Ivanova, M.O. Byrne, S.B. Milne, L.J. Marnett, H.A. Brown, Lipid profiling reveals arachidonate deficiency in RAW264.7 cells: structural and functional implications, Biochemistry 45 (2006) 14795–14808.
[144] C.A. Rouzer, P.T. Ivanova, M.O. Byrne, H.A. Brown, L.J. Marnett, Lipid profiling reveals glycerophospholipid remodeling in zymosan-stimulated macrophages, Biochemistry 46 (2007) 6026–6042.
[145] D. Balgoma, A.M. Astudillo, G. Pérez-Chacón, O. Montero, M.A. Balboa, J. Balsinde, Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids, J. Immunol. 184 (2010) 3857–3865.
[146] X. Han, R.A. Gubitosi-Klug, B. Collins, R.W. Gross, Alterations in individual molecular species of human platelet phospholipids during thrombin stimulation: electrospray ionization mass spectrometry-facilitated identification of the boundary conditions for the magnitude and selectivity of thrombin-induced platelet phospholipid hydrolysis, Biochemistry 35 (1996) 5822–5832.
[147] M. Valdearcos, E. Esquinas, C. Meana, L. Gil de Gómez, C. Guijas, J. Balsinde, M.A. Balboa, Subcellular localization and role of lipin-1 in human macrophages, J. Immunol. 186 (2011) 6004–6013.
[148] A.M. Astudillo, G. Pérez-Chacón, C. Meana, D. Balgoma, A. Pol, M.A. del Pozo, M.A. Balboa, J. Balsinde, Altered arachidonate distribution in macrophages from caveolin-1 null mice leading to reduced eicosanoid synthesis, J. Biol. Chem. 286 (2011) 35299–35307.
[149] P.T. Ivanova, S.B. Milne, H.A. Brown, Identification of atypical ether-linked glycerophospholipid species in macrophages by mass spectrometry, J. Lipid Res. 51 (2010) 1581–1590.
[150] J. Yang, S. Yang, X. Gao, Y.J. Yuan, Integrative investigation of lipidome and signal pathways in human endothelial cells under oxidative stress, Mol. Biosyst. (2011), doi:10.1039/c1mb00002k.
[151] E.A. Dennis, R.A. Deems, R. Harkewicz, O. Quehenberger, H.A. Brown, S.B. Milne, D.S. Myers, C.K. Glass, G. Hardiman, D. Reichart, A.H. Merrill Jr., M.C. Sullards, E. Wang, R.C. Murphy, C.R. Raetz, T.A. Garrett, Z. Guan, A.C. Ryan, D.W. Russell, J.G. McDonald, B.M. Thompson, W.A. Shaw, M. Sud, Y. Zhao, S. Gupta, M.R. Maurya, E. Fahy, S. Subramanian, A mouse macrophage lipidome, J. Biol. Chem. 285 (2010) 39976–39985.
[152] P.C. Norris, D. Reichart, D.S. Dumlao, C.K. Glass, E.A. Dennis, Specificity of eicosanoid production depends on the TLR-4-stimulated macrophage phenotype, J. Leukoc. Biol. (2011), doi:10.1189/jlb.0311153 jlb.0311153.
[153] A.Y. Andreyev, E. Fahy, Z. Guan, S. Kelly, X. Li, J.G. McDonald, S. Milne, D. Myers, H. Park, A. Ryan, B.M. Thompson, E. Wang, Y. Zhao, H.A. Brown, A.H. Merrill, C.R. Raetz, D.W. Russell, S. Subramaniam, E.A. Dennis, Subcellular organelle lipidomics in TLR-4-activated macrophages, J. Lipid Res. 51 (2010) 2785–2797.
[154] H.A. Brown, R.C. Murphy, Working towards an exegesis for lipids in biology, Nat. Chem. Biol. 5 (2009) 602–606.
[155] E. Fahy, S. Subramaniam, H.A. Brown, C.K. Glass, A.H. Merrill Jr., R.C. Murphy, C.R. Raetz, D.W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F. Spener, G. van Meer, M.S. Van Nieuwenhze, S.H. White, J.L. Witztum, E.A. Dennis, A comprehensive classification system for lipids, J. Lipid Res. 46 (2005) 839–861.