The Eicosanoid Research Division: Phospholipase A₂, Lipid Signaling and Lipidomics in Innate Immunity and Inflammation*

Jesús Balsinde

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

*http://www.balsinde.org
January 4, 2017

There is some discrepancy as to what really is the kick-off date of the Eicosanoid Research Division (or the Eicosanoid Laboratory, as it came to be known first). Truth be told, the thing took its first official steps in September 2000, with my definitive arrival in Valladolid. Beginnings are always difficult, and mine in the capital of the Old Castile was no exception. Because of this and a few other things, the details of which I will spare the reader, I normally set the beginning of our scientific adventures in Castile just one year after, in the fall of 2001, which is when something resembling a lab was finally put at my disposition and a few people were around to start the journey along. Our subject matter, obviously, lipids and all things lipids. Why always lipids?, you may ask. Good question. For the moment being, let’s say that lipids play fundamental roles in the regulation of cell signaling, and that imbalances in lipids and lipid-mediated signaling cause a large number of diseases, including type 2 diabetes, Alzheimer, arthritis and atherosclerosis. With that in mind, it should become obvious that one of the first steps we have to take in the direction of curing these diseases is to identify the lipids that are involved and to determine what they specifically do.

Many signaling lipids are generated by phospholipases such as phospholipase A₂, which produces lysophospholipids and free fatty acids like arachidonic acid. Further, many of the enzymes involved in the de novo pathways of lipid biosynthesis are also implicated in signaling. While our long-term goals include a comprehensive characterization of the roles played by both biosynthesis and remodeling pathways to overall cell signaling, our current research focuses primarily on the enzymes involved in maintaining the composition and levels of different fatty acids in biological membranes, i.e. phospholipases and acyltransferases. It is becoming clear that the phospholipid composition of biological membranes is very dynamic and that cells likely sustain many biological functions with different but somehow equivalent lipid compositions rather than a single one. Taking this concept a bit further, one could speculate that perhaps it is that, when the different lipid classes and subclasses interact to form a biological membrane, not all concentrations of each species are possible or permitted (or required). Something like quantized lipid states? Fascinating indeed. In our laboratory we combine a range of chemical, biochemical, pharmacological, and molecular cell biology techniques to study lipid metabolism and signaling in physiology and pathophysiology. Within this context, our current goals can be summarized into five points, which are described below. This report covers the research activities of our lab from the beginning until December 2016.

1) Cellular regulation of phospholipase A₂

Phospholipase A₂ enzymes catalyze a key reaction in signaling, i.e. the release of arachidonic acid and other polyunsaturated fatty acids from the sn-2 position of cellular glycerophospholipids. The products of phospholipase A₂ action, free fatty acids and lysophospholipids may act as signaling molecules on their own, and also serve as a precursors for the synthesis of platelet-
activating factor or the eicosanoids. Our group has a long history in studying the mechanisms of regulation of phospholipase A2 in resting and stimulated cells (reviews: 1-8). Our work has highlighted the differential contribution of various phospholipase A2s to the formation of cellular lysophospholipid pools, and the important role that group VIA phospholipase A2 plays in this process (9-11). This latter enzyme appears to serve multiple roles in cells, and delineating some of these has been the subject of our studies. Specifically, we have studied the involvement of group VIA phospholipase A2 in modulating lysozyme secretion as well as its various roles in cell proliferation and apoptosis (12-15). More recently, we have identified a hitherto unrecognized preference of this enzyme for choline phospholipids containing palmitic acid at the sn-1 position (16). Unveiling the biological functions and/or consequences that stem from such preference constitutes one of the focus of our current research.

Subcellular localization studies of various phospholipase A2 forms have also been conducted (17, 18). Various determinants and signaling events that regulate the subcellular localization of the arachidonate-specific group IVA phospholipase A2 have been described in detail. Among these, we have unveiled the role of a cationic cluster present in the catalytic domain of the protein in regulating membrane binding via interaction with anionic phospholipids. The involvement of phosphorylation reactions in regulating binding of the enzyme to phagosomes has been characterized as well (19-22). Further work has led to the identification of this enzyme as an early key factor for adipocyte differentiation in vitro, and in vivo during high fat diet-induced obesity (23).

Studies on group V secreted phospholipase A2 have shown that this enzyme is strongly upregulated in human macrophages by interleukin-4, but not by interferon-γ plus lipopolysaccharide. Further, we found that the increased expression of the enzyme in interleukin-4-treated macrophages serves to regulate the cellular levels of ethanolamine lysophospholipids that are necessary to support the elevated phagocytic response that these cells exhibit (24). These results raise the provocative idea that group V phospholipase A2 may act as a bi-faceted enzyme in innate immunity and inflammation, playing either pro- or anti-inflammatory roles depending on conditions, cell type and species.

(2) Signaling mechanisms involved in the biosynthesis of eicosanoids by cells of the immune system.

The eicosanoids are a large family of bioactive mediators that derive from the enzymatic oxygenation of arachidonic acid. The eicosanoids are biomedically important because they mediate all four signs of inflammation, namely heat, redness, swelling and pain. Controlling the formation of eicosanoids has been found to be of great benefit for the treatment of acute and chronic inflammatory diseases. Recently, we have characterized extensively the differences in arachidonate mobilization and eicosanoid metabolism by agonists acting via Toll-like receptors (25-28). Overall, our results provide support to a model whereby secreted group V phospholipase A2 may contribute to the eicosanoid biosynthetic response in some cases, by increasing activation of group IVA cytosolic phospholipase A2 through amplification of phosphorylation cascades. These studies are being extended to stimuli acting via other receptors, and also to the responses elicited by free fatty acids (29-32).

We have identified that lipin-1, a phosphatidate phosphatase enzyme residing on the surface of lipid droplets, may lie upstream group IVA phospholipase A2, thus providing new and unexpected ways of regulating arachidonate mobilization and eicosanoid synthesis (33). The lipin family of enzymes consists of 3 members, one of them exhibiting 3 splicing variants. Aside from the aforementioned role for lipin-1, it is not known whether other lipin proteins regulate the eicosanoid cascade and we are conducting studies to explore this possibility. Importantly, these enzymes appear to play significant roles not only in regulating phospholipid metabolism (13) but also in key aspects of innate immunity and inflammation such as lipopolysaccharide signaling and systemic inflammation (34), and activation of the NLRP3 inflammasome (35).

(3) Membrane fatty acid metabolism; incorporation into phospholipids and remodeling.

The distribution of polyunsaturated fatty acids in cells is achieved by a finely regulated set of reactions of incorporation, remodeling and liberation of the fatty acids into, among and from phospholipids, respectively. These reactions ensure the proper distribution of the fatty acids within the various cellular phospholipid pools, which is important not only for membrane homeostasis but also for the execution of appropriate cell responses during physiological and pathophysiological activation. Our current work pays special attention to the interactions between omega-3 and omega-6 fatty acids, due to their
importance as precursors of a large number of bioactive substances. Members of these two fatty acid families compete with each other for incorporation into the sn-2 position of membrane glycerophospholipids, which provides a control point for regulating their cellular levels, and hence the amount of oxygenated products generated after stimulation. Our work has led to the discovery of the selective involvement of lysophosphatidylcholine acyltransferase 3 (LPCAT3) as a novel signal-regulated enzyme involved in arachidonic acid metabolism in human monocytes (36). Also, inhibition studies of phospholipid fatty acid incorporation and remodeling at various points have shown elevations of cellular free arachidonate levels that alter the balance of eicosanoids produced (16) and may also conduce to cell death by apoptosis (37). Ongoing studies aim at characterizing at the cellular level the different specificities of the CoA-dependent routes of fatty acid incorporation (acyl-CoA synthetases and CoA-dependent acyltransferases), as well as the subsequent redistribution of the fatty acids into various cellular pools by CoA-independent transacylases (38, 39). Collectively, our research in this area supports the concept that differential incorporation (acyl-CoA synthetases and CoA-dependent acyltransferases), as well as the subsequent redistribution of the fatty acids into various cellular pools by CoA-independent transacylases (38, 39). Collectively, our research in this area supports the concept that differential stimulation of phagocytic cells promotes selective lipid turnover and, therefore, the appearance of specific lipid signatures for each activation condition.

(4) Biosynthesis and degradation of lipid droplets during cellular activation.

Lipid droplets are dynamic cytoplasmic structures which, among many other things, may function as docking platforms for a number of enzymes involved in lipid signaling (8). We have recently described the regulation of lipid droplet biosynthesis by various members of the phospholipase A2 family of enzymes as well as the differential involvement of various lipid metabolic pathways in signal-regulated lipid droplet formation under proinflammatory conditions (40-43). Our most significant results in this area of research suggest that phosphorylation activation of group IVA phospholipase A2 by multiple kinases drives lipid droplet formation in cells possibly by facilitating biogenesis of this organelle, not by regulating neutral lipid synthesis (43).

In defining the origin of the fatty acids accumulating in the neutral lipids of lipid droplets under a variety of stimulatory conditions (i.e. increased de novo fatty acid synthesis versus mobilization from membrane phospholipids) we made the unexpected finding that the lipid droplets of activated monocytes are enriched with a very unusual fatty acid, cis-7-hexadecenoic acid (16:1n-9), a positional isomer of palmitoleic acid. The fatty acid shows significant anti-inflammatory activity in vitro and in vivo and may be a biomarker for early detection of cardiovascular disease (44). Since cis-7-hexadecenoic acid accumulates in foamy monocytes in response to free arachidonic acid, a pro-inflammatory mediator, it is conceivable that metabolic changes underlying the formation and accumulation cis-7-hexadecenoic acid fatty acid into specific lipid classes are instrumental in showcasing effector functions of monocytes and macrophages toward the re-establishment of homeostasis during the course of inflammation processes. Work in progress aims at understanding the biochemical pathways, cellular regulation, mechanisms of action, and spectrum of biological activity in vivo of cis-7-hexadecenoic acid.

(5) Lipidomics and metabololipidomics; identification and quantification of cellular lipidomes by mass spectrometry.

The development of mass spectrometry techniques for the detection and analysis of lipids at a global scale provides us with a unique opportunity to characterize in detail the changes occurring in lipid metabolism as a consequence of cell activation (Reviews: 6, 7). Since multiple lipid metabolic pathways are known to be activated by receptor stimulation of cells, one of the major goals of our research in this area is to determine the origin and identity of the individual phospholipid molecular species that are produced under different conditions, as a first step to address their biological roles in cells. In the context of these studies, we have described a number of novel arachidonate-containing lipids, the levels of which increase during cell activation (45, 46). A remarkable finding from these studies was the discovery of the novel molecular species 1,2-di(arachidonoyl-sn-glycero-3-phosphoinositol as a major but transient reservoir of arachidonic acid. The molecule appears to be produced by sequential remodeling of a preexisting phosphatidylinositol molecule first at position sn-2 and then at sn-1 with arachidonate, and could be involved in modulating innate immune responses such as superoxide anion production or secretion of antimicrobial hydrolases (29).

Other arachidonate-containing lipids have been identified in a stimulus- and cell type-dependent manner. A potentially interesting one is 1-palmitoleyl-2- arachidonoyl-sn-glycero-3-phosphoethanolamine, which is not present in resting monocytes but accumulates
rapidly under receptor stimulation (46). Again, these studies support the idea that changes in lipid content upon activation may be stimulus-specific, and also that cellular activation includes both common and stimulus-specific markers. Similar work has been conducted with lipids containing arachidonic acid, the two-carbon elongation product of arachidonic acid (47). Our data in this regard suggest that significant differences exist between the cellular mechanisms regulating the availability of arachidonic acid and its immediate precursor arachidonic acid that could potentially be exploited to design strategies to control the production of oxygenated products of both fatty acids.

Finally, our expertise in lipidomics has made it possible for us to interact with other research groups, and these interactions have led to collaborative studies that focus on the identification of lipid alterations in various models of disease, including caveolin-1 deficiency (39, 48-50), hypertriglyceridemia (51), liver disorders (52-55), cancer (56), and spinal cord injury (57). Several other lipidomic analyses are currently being carried out in our laboratory under these collaborative agreements.

REFERENCES


