

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

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December 10, 2013

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 old capital of Castile was no exception. Because of this and a few other things, the details of which I will spare the reader, I usually like to 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 just 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 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 the maintenance of the

composition and levels of different fatty acids in biological membranes, i.e. phospholipases and acyltransferases. 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 four points, which are described below. (This report covers the research activities of our lab from the beginning until December 2013).

(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 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: 1-5). Our recent 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 (6-8). 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 (9-12).

Subcellular localization studies of the various phospho-

lipase A₂ forms have also been conducted (13, 14), and various determinants and signaling events that regulate the subcellular localization of the arachidonate-specific group IVA cytosolic 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 (15-18).

(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 (19-22). 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 VIA cytosolic phospholipase A₂ through amplification of phosphorylation cascades. These studies are being extended to agonists acting via other innate immune receptors, and also to the responses elicited by free fatty acids (23-25).

(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 (26). 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 (24) and may also conduce to cell death by apoptosis (27). 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 (28, 29). 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 (30). We have recently described the regulation of lipid droplet biosynthesis by various members of the phospholipase A₂ family of enzymes (31-33) as well as the differential involvement of various lipid metabolic pathways in signal-regulated lipid droplet formation under proinflammatory conditions). 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 (34). Current work aims at precisely defining the origin of the fatty acids accumulating in lipid droplets under a variety of stimulatory conditions (i.e. increased *de novo* fatty acid synthesis *versus* mobilization from membrane phospholipids).

(5) Lipidomics and metabolipidomics; 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: 35, 36). Since multiple lipid metabolic pathways are known to be activated via receptor stimulation in 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 (37, 38). A remarkable finding from these studies was the discovery of the novel molecular species 1,2-diarachidonoyl-*sn*-glycero-3-phosphoinositol as a major but transient reservoir of arachidonic acid. We are currently investigating the regulation of metabolic pathways and enzymes involved, as well as possible biological processes mediated by this species. Our most recent results suggest that the molecule is produced by sequential acylation of positions *sn*-2 and then *sn*-1 by remodeling with arachidonate of a preexisting phosphatidylinositol molecule, and could be involved in modulating innate immune responses such as superoxide anion production or secretion of antimicrobial hydrolases (23)

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, a phospholipid that is not present in resting monocytes but accumulates rapidly under receptor stimulation (38). Once 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 (23, 37, 38). Similar work has been initiated with lipids containing adrenic acid, the two-carbon elongation product of

arachidonic acid (39). Our data in this regard suggest that significant differences exist between the cellular mechanisms regulating the availability of adrenic 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. We plan on extending these studies to lipids containing omega-3 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 (28, 40, 41), hypertriglyceridemia (42), alcohol-induced liver disease (43), and cancer (44). Several other lipidomic analyses are currently being carried out in our laboratory under these collaborative agreements.

Future Directions

As discussed briefly above, one of our near-future goals is to extend our lipidomic studies to omega-3 fatty acids. We will identify all the cellular lipids that contain omega-3 fatty acyl esters and will follow their concentrations under innate immune activation conditions. These studies should be complemented with work utilizing exogenous fatty acids to verify whether omega-3 fatty acyl lipid esters undergo time-dependent remodeling similar to that of omega-6 fatty acids, and to define the newly-formed species that may accumulate under the different cell treatments.

In subsequent work we plan on studying cellular signaling that may involve omega-3 fatty acids, to define new molecules and lipid pathways with anti-inflammatory potential. One of these could well be the lipin-2-mediated pathway recently described by us (25). A second route that we expect to examine in relation to omega-3 fatty acid signaling is the phospholipase D pathway. After so many years away from the phospholipase D field, working again on this enzyme (45-48) could rightfully be seen as taking a sentimental journey to the past!

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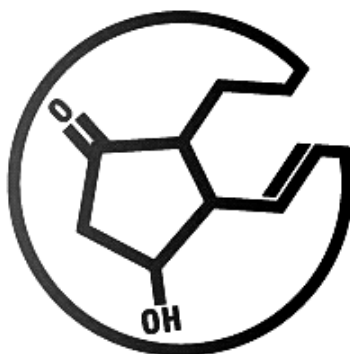
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