Multidisciplinary Approaches to the Study of Lipid Signaling

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Lipids are essential for the regulation of cell signaling. Hence they are key to maintaining our homeostatic processes. Not surprisingly, imbalances in lipid metabolism cause an ample number of pathologies. For us to be able to treat these diseases with success, we need in the first place to identify the lipids involved and what they do. In our laboratory we combine a range of chemical, biochemical, pharmacological, and molecular cell biology techniques to study pathophysiologically-relevant problems involving alterations in lipid metabolism and signaling. The ultimate goal is to identify and characterize novel lipid pathways responsible for disease.

Numerous signal transduction processes involve lipids as signaling molecules. Many of these molecules are generated by phospholipases such as phospholipase A_2, which releases fatty acids like arachidonic acid, and lysophospholipids. Each of these products is implicated in signal transduction processes, but also serves as a precursor for platelet activating factor or the eicosanoids. The eicosanoids are a large family of bioactive mediators that derive from the enzymatic oxygenation of arachidonic acid. Prostaglandins, leukotrienes, thromboxane, lipoxins, are all members of the eicosanoid family. Circulating monocytes and tissue macrophages are major sources of these compounds. 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.

Lipid signaling is also key to the development of cardiovascular disease, one of the most prevalent inflammatory disorders. Atherosclerosis is the primary cause for cardiovascular disease, and diabetes increases the risk several-fold by enhancing the formation and/or progression of atherosclerotic lesions, a process in which abnormally-activated monocytes and macrophages appear to play a major role. In diabetes, these cells appear to be in a proinflammatory state, releasing elevated amounts of cytokines and eicosanoids that perpetuate the inflammatory condition. Monocytes/macrophages from diabetic patients have been found to exhibit enhanced expression of Toll-like receptors 2 and 4. These receptors sense bacterial pathogens but also endogenous danger molecules such as saturated free fatty acids, typically present at elevated amounts in obese individuals.

Our current research focuses primarily on the lipid signaling enzymes phospholipase A_2 and phosphatidate phosphohydrolase (phosphatidic acid-specific phospholipase C; lipin). The latter is a key enzyme in the de novo pathway for glycerolipid biosynthesis, providing an excellent example that enzymes involved in this pathway may also act to initiate intracellular signaling. General events that we are interested in include (i) the spatiotemporal regulation of these phospholipases in a cellular context, which we study utilizing advanced microscopy techniques, (ii) pharmacological manipulation of enzymatic activity both in intact cells and in vitro, (iii) analysis of lipid metabolite production by state-of-the-art mass spectrometry (lipidomics & metabolipidomics), and (iv) the physiological functioning of phospholipases in animal models.

Ongoing studies in our labs focus on the localization and stimulus-driven translocation of different members of the phospholipase A_2 and lipin families. Phospholipase A_2s cleave the fatty acid at the sn-2 position of phospholipids and thus constitute the earliest regulatory point of the eicosanoid biosynthetic cascade. Lipins dephosphorylate

phosphatidic acid to form diacylglycerol, which can be used for the biosynthesis of glycerophospholipids and triacylglycerol, and may function as intracellular signalers as well. Current studies are being carried out by transfecting chimeric constructs of green fluorescent protein (GFP) (or any of its colored varieties) with the appropriate phospholipase. GFP is placed at either the N- or C-termini. of the enzymes. These constructs provide a very useful tool to visualize the intracellular movements of the enzymes in response to the different stimuli. Mutagenesis studies are also being conducted to pinpoint the specific amino acids of the phospholipase A_2s and lipins that are implicated in the movement among intracellular compartments.

Another of our goals is to apply a lipidomics approach to the study of the mechanisms governing the availability and oxidative metabolism of free arachidonic acid during activation of macrophages by stimuli of the innate immune response. Availability of free arachidonate is a limiting step for the synthesis of eicosanoids. While the pathways of fatty acid uptake, incorporation and remodeling in glycerolipids are well documented, the individual lipid species in which arachidonate is stored and released from have not been identified. This is so because of the impossibility of traditional methods for lipid separation (i.e. thin-layer chromatography, liquid chromatography) to differentiate among individual lipids within various classes and subclasses. This is now possible with the advent of electrospray mass spectrometry (ESI-MS). Application of this technology to the field of lipid biochemistry has been a major breakthrough in profiling the lipidomes of cells and tissues in physiological and pathophysiological conditions. We are conducting lipidomic analyses of all the lipid molecular species involved in arachidonic acid homeostasis, from those that act as acceptors of the fatty acid to those from which the fatty acid is liberated for subsequent eicosanoid synthesis, and including as well a full survey of arachidonate-derived oxygenated metabolites. In the context of these studies, we have described a number of novel arachidonate-containing lipids, the levels of which increase during cell activation. We are currently investigating the metabolic pathways involved in their biosynthesis as well possible biological processes mediated by these species.

Finally, we have begun studies aimed at defining the regulation of lipid droplet formation in cells involved in inflammation. Lipid droplets are cytosolic inclusions present in most eukaryotic cells that contain a core rich in neutral lipids such as triacylglycerol and cholesteryl esters and are surrounded by a phospholipid monolayer decorated with a variety of proteins. Initially regarded as inert neutral lipid-storage compartments, the interest for lipid droplets has increased recently because of their association with diabetes and atherosclerosis. Our results have defined group IVA phospholipase A_2 as a key regulator of lipid droplet formation. Also, subcellular localization studies have shown that lipin-1 localizes permanently on the surface of these organelles, thus suggesting a metabolic or regulatory role for this enzyme. Lipidomic analyses of the composition of lipid droplets formed under various conditions, have uncovered the presence of unusual fatty acids in these organelles. Some of these fatty acids might play a role in regulating specific cellular responses.

All of our lines of research rely heavily on biochemical and analytical methods to identify specific reactions and the mechanisms through which the products of said reactions are formed. With this information, we expect to delineate pathways responsible for disease. In summary, in our laboratory we combine a range of chemical, biochemical, pharmacological, and molecular cell biology techniques to study pathophysiologically-relevant problems involving alterations in lipid metabolism and signaling.

(Updated Results & Publications sections – The Eicosanoid Research Division – www.balsinde.org)

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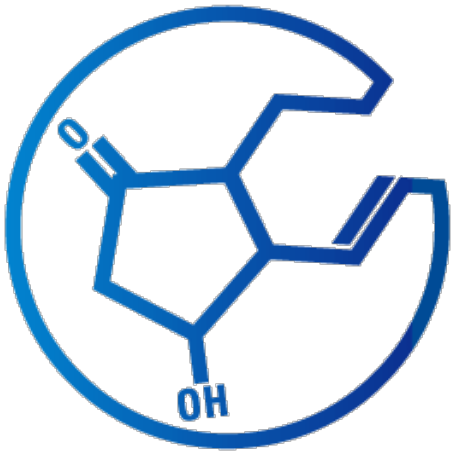

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