The Eicosanoid Research Division: Phospholipase A_2, Lipid Signaling and Lipidomics*

RESEARCH SUPPORT HISTORY

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
December 3, 2020

This document lists the research grants obtained by J.B. as principal investigator from 2001 to the date listed above. It will be updated as appropriate. Only grants from competitive calls, whether national or regional, public or private, are included in this document.

(1) **Intracellular Signaling Mechanisms Regulating Prostaglandin Biosynthesis in Immunoinflammatory Cells (Spanish Ministry of Science and Technology, ref. BMC2001-2244) (2001-2004)**

The prostaglandins are a family of oxygenated derivatives of arachidonic acid that potently mediate a wide variety of physiological and pathophysiological processes, most notably those of inflammatory nature. The goal of the current project proposal is to increase our knowledge on the molecular mechanisms that govern prostaglandin biosynthesis. This project focuses on the characterization of those intracellular regulatory systems that modulate the expression and/or activity of phospholipases and cyclooxygenases, i.e. the final effectors of the prostaglandin biosynthetic response. This project consists of three main interrelated objectives, as follows: 1) expression levels of group V phospholipase A_2; 2) role of cell activation-induced plasma membrane alterations (asymmetric movement of phospholipids and importance of membrane rafts); 3) novel regulatory pathways mediated by polyphosphoinositides.

Publications derived from this grant (1-10).

(2) **Signal Transduction Mechanisms Involved in the Activation of Phagocytic Cells (Regional Government of Castile and León, Department of Education, ref. CSI 4/02) (2002-2004)**

The prostaglandins are a family of oxygenated derivatives of arachidonic acid that potently mediate a wide variety of physiological and pathophysiological processes, most notably those of inflammatory nature. The main goal of the current project is to increase our knowledge on the molecular mechanisms that govern prostaglandin biosynthesis by immunocompetent cells. This project consists of two objectives, namely: 1) expression levels of group V phospholipase A_2. This enzyme is involved in generating the prostaglandin metabolic precursor free arachidonic acid; 2) role of cell activation-induced plasma membrane alterations (asymmetric movement of phospholipids and importance of membrane rafts) on phospholipase A_2 activation.

Publications derived from this grant (2-8).

(3) **The Lipid Hypothesis in Schizophrenia, a Novel Therapeutic Approach. Inhibitors of Calcium-independent Phospholipase A_2: Synthesis, Enzyme Inhibition, and Signal Transduction (Fundació La Marató de TV3, ref. 011232) (2002-2004)**

The overall goal of this research proposal is the generation of novel inhibitors of calcium-independent phospholipase A_2, as well as the analysis of the impact of these inhibitors on lipid metabolism in brain. We propose three objectives: (i) chemical synthesis, (ii) biochemical characterization of the inhibitors, and (iii) effect of these compounds on cell signaling mediated by the nuclear receptors PPARγ and RXR through activation of MAP kinases. Different lines of evidence have suggested that signal transduction

pathways involving retinoid and polyunsaturated fatty acid signaling may be important factors in the ethiology of schizophrenia. Release of polyunsaturated fatty acids from phospholipids is mediated by phospholipase A_2 enzymes, some of which may be augmented in schizophrenic patients. Our working hipothesis, based on the very well known role of phospholipase A_2 in signal transduction is that inhibition of this class of enzymes may contribute to prevent signaling leading to depletion of polyunsaturated fatty acids, a hallmark of schizophrenia.

Publications derived from this grant (2-10,11).

(4) **Regulation of Cyclooxygenase-2 Expression by Phospholipase A_2-derived Lipid Products (Spanish Ministry of Education and Science, ref. BFU2004-01886/BMC) (2004-2007)**

The eicosanoids are derivatives of arachidonic acid that play important roles in inflammation by mediating the signs that are strikingly associated to the illness. Inflammation is a hallmark of numerous pathologies, ranging from septic shock to rheumatoid arthritis or Alzheimer's Disease. Thus, it is important to find new molecular targets to produce anti-inflammatory drugs with improved selectivity and, as much as possible, devoid of side-effects. Group V secreted phospholipase A_2 as an enzyme responsible for generating free arachidonic acid to be used for the biosynthesis of prostaglandins. In addition, group V phospholipase A_2 regulates the expression of the cyclooxygenase-2 (COX-2) gene itself. This regulation appears to take place through the production of an unidentified metabolite downstream of phospholipase A_2 activation. In the present grant project we plan to study: 1) the molecular nature of the metabolite responsible for COX-2 gene expression; 2) the cellular regulation of the enzymes involved in the synthesis of this metabolite; 3) changes in gene expression utilizing microarray techniques; and 4) identify the cellular targets of this metabolite.

Publications derived from this grant (8-18).

(5) **Regulation of Cyclooxygenase-2 Expression and Activity in Alzheimer's Disease (Fundación La Caixa, ref. BM05-248-0) (2005-2008)**

Prostaglandins and other lipid mediators regulate key aspects of neural membrane biology in the central nervous system. However overproduction of these substances may cause cellular injury. Prostaglandins derive from the enzymatic oxygenation of arachidonic acid, a fatty acid that is released from its phospholipid storage sites by phospholipase A_2. Disregulated phospholipase A_2 activity has been correlated with several forms of acute and chronic brain injury, including cerebral trauma, cerebral ischaemia, epilepsy, schizoprenia, and in particular, Alzheimer's Disease. The expression of both phospholipase A_2 and cyclooxygenase-2 activities is strongly up-regulated during Alzheimer's Disease, indicating the importance of inflammatory gene pathways as a response to brain injury. Previous studies from the applicants have established that phospholipase A_2 not only provides the substrate for cyclooxygenase-2 to act upon (i.e. free arachidonic acid), but also controls cyclooxygenase-2 gene induction through generation of a metabolite of unknown structure. Backed up by our experience in this area of research, we propose: (1) to establish the identity of the compound that is responsible for cyclooxygenase-2 gene induction; (2) to study the molecular regulation of the enzymes involved in its synthesis; (3) to study the expression of putative cellular targets for the aforementioned compound by microarray technology; and (4) to study the molecular regulation of these targets during an inflammatory injury. These studies will be conducted on microglial cells and astrocytes and will eventually allow us to establish trends to look for molecular targets against which to develop new drugs with anti-inflammatory potential that can be used in the treatment of chronic inflammatory diseases of the brain such as Alzheimer's Disease.

Publications derived from this grant (16-18).

(6) **A Lipidomics Approach to the Study of the Innate Immune Response: Mechanisms Governing Arachidonic Acid Availability and Metabolism in Macrophages (Spanish Ministry of Science and Technology, ref. BFU2007-67154/BMC) (2007-2010)**

Inflammatory diseases bear strong social repercussions because of the elevated number of individuals suffering from them and the high costs of heath care involved. Illnesses clearly involving an inflammatory component include rheumatoid arthritis, stroke, metabolic syndrome, neurological disorders such as Alzheimer's disease, and some types of cancer. A common feature of all of these illnesses is the existence of imbalances in lipid metabolic pathways. On the other hand, the innate immune system plays key regulatory roles in the initiation, development and resolution of inflammatory diseases. Lipids contribute quite significantly to the pool of bioactive mediators of the inflammatory response, in particular those derived from

enzymatic oxygenation of arachidonic acid (AA). Although much advance has recently been made in the understanding of biochemical pathways and biological actions of proinflammatory lipid mediators, technological limitations have greatly difficulted the accurate characterization of species that typically occur in quantities as low as pmoles. The introduction of electrospray mass spectroscopy to the lipid field has been the major breakthrough that now makes it possible to profile the lipidomes of cells and tissues under a variety physiological and pathophysiological situations. The present research proposal focuses on the application of a mass spectrometry-based lipidomics approach to the study of AA availability and oxidative metabolism in macrophages, the innate immune cells by excellence. We propose the following aims: (i) to establish the profile of eicosanoid metabolites (eicosanomes) produced under stimulation with various stimuli of the innate immune response, (ii) to identify the individual phospholipid species from which AA is released during proinflammatory stimulation, (iii) to determine the individual lysophospholipid acceptors involved in the reacylation reactions, and (iv) to study sphingolipid metabolism during apoptotic cell death induced by excess free AA. Completion of these aims will increase our knowledge of the regulatory features of AA homeostasis during innate immunity and inflammation and help identify novel molecular targets for pharmacological intervention in treating these diseases with an inflammatory component.

Publications derived from this grant (18-30).

(7) **Role of Calcium-independent Phospholipase A_2 in Oxidative Stress and Apoptosis (Regional Government of Castile and León, Department of Education, ref. CSI09A08) (2008-2010)**

Oxidative damage is a pathophysiological condition that accompanies a variety of inflammatory states. Phagocytic cells produce substances with high oxidant capacity during inactivation and phagocytosis of invading pathogens. However, an uncontrolled production of these oxidants may lead to damage and hence, may constitute a very serious problem for the host. Oxidative damage usually occurs in parallel with the mobilization of free fatty acids such as arachidonic acid (AA) from membrane phospholipids. It is likely that these two processes are causally related, although the mechanisms involved are not understood. The current research proposal focuses on the elucidation of the molecular mechanisms through which phospholipase A_2 activity is augmented during oxidative stress and the apoptotic processes that ordinarily ensue. Previous work by our group has shown that calcium-independent phospholipase A_2 ($iPLA_2$) is responsible for the mobilization of free fatty acids during oxidative stress. Based on these previous findings, we propose to study: (i) the molecular nature of the oxidized phospholipid species that are produced during cellular exposure to hydrogen peroxide, and their potential effects on $iPLA_2$; (ii) the molecular mechanisms associated to hydrogen peroxide-induced apoptosis; (iii) changes in $iPLA_2$ activity and/or physical state that may account for its enhanced capacity to destroy membrane phospholipid; (iv) the role of $iPLA_2$-derived products, free fatty acids and lysophospholipids, during oxidant-induced apoptosis; and (v) the role of $iPLA_2$ on the phagocytosis of apoptotic cells by phagocytes.

Publications derived from this grant (22, 23, 25-30).

(8) **Mass Spectrometry Lipidomic Profiling of Human Macrophage Polarization (Spanish Ministry of Science and Education, ref. BFU2010-18826/BMC) (2011-2013)**

Macrophages are innate immune cells with well established roles in the primary response to pathogens, but also in tissue homeostasis, coordination of the adaptive immune response, inflammation, resolution, and repair. Initial responses to pathogens are mediated by engagement of innate immune receptors on the surface of macrophages, most of which are coupled to the generation of arachidonic acid-derived mediators (i.e. the eicosanoids). Although much progress has recently been made in understanding the role of bioactive lipids in mediating innate immune reactions, very little is known on the regulation by lipids of adaptive immune responses triggered by polarized M1 or M2 macrophages. Polarized activation of macrophages is critically determined by cytokine microenvironment, and endows the cells with highly specialized functional properties. The present research proposal focuses on the application of a mass spectrometry-based lipidomics approach to establish the lipidome of polarized M1 and M2 macrophages, with particular attention to the eicosanome (arachidonate-derived eicosanoids) and arachidonome (cellular arachidonate-containing lipids). With this information, molecular "fingerprints" of each macrophage state will be obtained that permits the identification of specific traits of the adaptive immune response in terms of lipid metabolic pathways involved. We propose the following aims: (i) to

conduct a global lipidomic profiling of polarized M1 and M2 macrophages, (ii) to apply a metabolipidomic approach to study fatty acid uptake and esterification processes in polarized macrophages, (iii) to establish the profile of AA oxygenated metabolites produced by polarized macrophages, (iv) to identify phospholipid sources for AA mobilization, and (v) to study changes in the expression of AA-metabolizing enzymes. Completion of these aims will increase our knowledge of key regulatory aspects of the adaptive immune response and may help identify new molecular markers and signatures which represent candidate targets with potential for pharmacological intervention.

Publications derived from this grant (30-47).

(9) **Mechanisms governing the availability and oxidative metabolism of arachidonic acid in human macrophages: a lipidomic study (Regional Government of Castile and León, Department of Education, ref. CSI007U13) (2013-2014)**

Inflammatory-based illnesses such as diabetes and cardiovascular disease bear strong social repercussions because of their high prevalence. Cells of the innate immunity plays a role in the initiation and resolution of these processes. Pro- and anti-inflammatory lipid derivatives of arachidonic acid (AA) play key roles in the inflammatory response. Not until recently, the scientific community has had the appropriate methodology for the study of these substances. The use of mass spectrometry as a tool for analysis of the species lipid cell (lipidomas) has opened a huge universe in the study of these metabolites. This project focuses on the application of mass spectrometry to the study of the availability of AA in macrophages, cells of the innate immune system par excellence, during inflammatory processes. Specific objetives of this proposal include: (i) to define the full lipidomic profile of phospholipids and neutral lipids of human macrophages; (ii) to identify the lysophospholipids lisofosfolipids that incorporate AA during the immune response via reacylation reactions; (iii) to establish the profile of AA oxygenated metabolites produced by macrophages in response to different stimuli of the innate immune response; and (iv) yo identify the species of phospholipids from which of the AA is released. This will allow a better understanding of lipid species that are involved in the innate innune response, allowing the identification of new therapeutic targets for the control of diseases with a clear inflammatory component.

Publications derived from this grant (46,47).

(10) **Anti-inflammatory lipid pathways regulating activation of the inflammasome: roles of omega-3 fatty acids and lipin-2 (Spanish Ministry of Economy and Competitiveness, ref. SAF2013-48201-R) (2014-2016)**

The inflammasome is an intracellular multiprotein complex responsible for generating IL-1β, which participates in the destruction of pathogenic microorganisms. It is activated by pathogen-associated molecular patterns and also by endogenous molecules generated by the body during situations of risk or damage. Because of the latter, inflammasome activation is also central to the development of nonmicrobial diseases such as type 2 diabetes, rheumatoid arthritis and atherosclerosis. The general objective of this project is to define novel routes for the involvement of omega-3 fatty acids in the inhibition of inflammasome activation via generation of lipids containing omega-3 fatty acyl esters, as well as to establish the molecular mechanisms by which lipin-2 dampens inflammasome activation, and verify whether they involve generation of these novel omega-3 lipid molecules. The proposal is divided into four specific objectives which are defined as follows: (i) to establish the omega-3 lipidome of human monocytes and macrophages, (ii) to isolate and/or chemically synthesize/derivatize potentially interesting omega-3 lipid esters and deliver them into cells, (iii) to explore the biological role of specific omega-3-contining lipid esters within anti-inflammatory pathways, and (iv) to define novel anti-inflammatory routes regulated by lipin-2, potentially involving omega-3 lipid esters. Overall we will generate new data to better understand the biology of the inflammasome and its implications in pathophysiology. Our work will define new omega 3-derived molecules and pathways involved in the inhibition of inflammasome, the manipulation of which could be used for the development of therapeutic strategies for the treatment of inflammatory conditions where the inflammasome plays a central role.

Publications derived from this grant (46-56).

(11) **Positional isomers and oxidized derivatives of palmitoleic acid as novel mediators of inflammation (Regional Government of Castile and León, Department of Education, ref. CSI073U16) (2016-2018)**

Recent studies have put palmitoleic acid, an n-7 monounsaturated fatty acid, in the spotlight of inflammatory lipid research owing to its protective effect on hepatic steatosis and improvement of insulin signaling

in murine animals of metabolic disease. Thus, palmitoleic acid has been defined as a lipid hormone that coordinates metabolic crosstalk between liver and adipose tissue. However, studies performed in human subjects have provided contradictory outcomes. A major problem in this area is that the mechanism of action and active form of palmitoleate are unknown. Moreover, the direct actions of palmitoleate on immunoinflammatory cells remain largely unexplored. A consistently ignored fact is that palmitoleic acid isomers do occur in mammalian cells. It could be that the multiplicity of effects and the compartmentalized manner in which the palmitoleic acid effects are reported to occur is due to the overlapping actions of such isomers being present at the same or neighboring locations. The present research proposal is articulated around three major objectives, all of them exploring completely uncharted territory, that should provide key information to understand the physiological and pathophysiological implications of palmitoleic acid and its isomers. These objectives are: (i) to establish the occurrence of palmitoleic acid isomers in human monocytes and macrophages, and the biochemical pathways for their synthesis and compartmentalization, (ii) to analyze the formation of hydroxylated derivatives of palmitoleic acid and its isomers, and (iii) to develop strategies and assays to unveil the bioactive form of palmitoleic acid. Completion of these aims will ultimately result in the full characterization of the active form of the palmitoleic acid that is responsible for the variety of biological activities ascribed to this fatty acid, and may open up new horizons and opportunities for the development of new strategies to treat inflammatory and metabolic diseases.

Publications derived from this grant (57-60).

(12) **Molecular mechanisms of action and in vivo activity of a novel anti-inflammatory fatty acid, cis-7-hexadecenoic acid (16:1n-9) (Spanish Ministry of Economy, Industry and Competitiveness, ref. SAF2016-80883-R) (2017-2019)**

Excessive or prolonged inflammation is a key cause for the development and persistence of many disorders, including those of metabolic origin. Lipid metabolism is known to be at the center of many of these diseases, and it is now appreciated that lipid mediators are produced during the course of inflammatory reactions in two temporally distinct waves with opposite effects, such that the cells switch the type of mediators produced from pro- to anti-inflammatory. This process, termed “class switching”, initiates resolution of inflammation and the return to homeostasis. Thus cells intrinsically possess programed mechanisms to dampen inflammation so as to avoid excessive damage that might lead to irreversible injury. In this regard, our previous work demonstrated that potent pro-inflammatory lipids such as free arachidonic that are released by cells at various sites of injury, may act on innate immune cells to ultimately promote the synthesis and accumulation in these cells of an unusual fatty acid with opposing, anti-inflammatory effects, preventing in this manner excessive damage. This fatty acid, unambiguously identified by mass spectrometry as cis-7-hexadecenoic acid (16:1n-9), was found primarily esterified in neutral lipids within cytoplasmic lipid droplets of human monocytes. This distribution is in stark contrast with that of all other fatty acids of human monocytes, which localize primarily in membrane phospholipids. Based on these previous observations, it is conceivable that metabolic changes underlying the formation and accumulation of this novel 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. The present research proposal is articulated around four specific objectives that should provide key information to understand the biochemical pathways, cellular regulation, mechanisms of action, and spectrum of biological activity in vivo of 16:1n-9. These objectives are formulated as follows: (i) to characterize the full lipidome of species containing 16:1n-9: dynamics of incorporation into and remodeling between cellular lipids during inflammatory activation; (ii) to chemically synthesize/ derivatize potentially interesting lipids containing 16:1n-9 moieties and deliver them into cells; (iii) to delineate the signaling pathways and molecular mechanisms involved in the anti-inflammatory effects of 16:1n-9; and (iv) to define the role of 16:1n-9 on inflammatory-based metabolic diseases by using animal models. Completion of this proposal will lay a solid foundation for understanding, at a molecular level, new concepts on anti-inflammatory mechanisms in a number of diseases that share inflammation as a common ethiopathogenic factor, and permit the identification of master regulators of this novel class switching involving 16:1n-9 that may open the door to future therapeutic manipulation.

Publications derived from this grant (54-69).

(13) **Novel lipid mediators of inflammation: palmitoleic acid isomers and derivatives (Spanish Ministry of Economy and Competitiveness, ref.**

SAF2015-73000-EXP) (2017-2019)

Recent studies have put palmitoleic acid, an n-7 monounsaturated fatty acid, in the spotlight of inflammatory lipid research owing to its protective effect on hepatic steatosis and improvement of insulin signaling in murine animals of metabolic disease. Thus, palmitoleic acid has been defined as a lipid hormone that coordinates metabolic crosstalk between liver and adipose tissue. However, studies performed in human subjects have provided contradictory outcomes. A major problem in this area is that the mechanism of action and active form of palmitoleate are unknown. Moreover, the direct actions of palmitoleate on immunoinflammatory cells remain largely unexplored. A consistently ignored fact is that palmitoleic acid isomers do occur in mammalian cells. It could be that the multiplicity of effects and the compartmentalized manner in which the palmitoleic acid effects are reported to occur is due to the overlapping actions of such isomers being present at the same or neighboring locations. The present research proposal is articulated around three major objectives, all of them exploring completely uncharted territory, that should provide key information to understand the physiological and pathophysiological implications of palmitoleic acid and its isomers. These objectives are: (i) to establish the occurrence of palmitoleic acid isomers in human monocytes and macrophages, and the biochemical pathways for their synthesis and compartmentalization, (ii) to analyze the formation of hydroxylated derivatives of palmitoleic acid and its isomers, and (iii) to develop strategies and assays to unveil the bioactive form of palmitoleic acid. Completion of these aims will ultimately result in the full characterization of the active form of the palmitoleic acid that is responsible for the variety of biological activities ascribed to this fatty acid, and may open up new horizons and opportunities for the development of new strategies to treat inflammatory and metabolic diseases.

Publications derived from this grant (57-60).

(14) **Novel phospholipid signatures involved in metabolic activation of macrophages by saturated fatty acids (Spanish Ministry of Science and Innovation, ref. PID2019-105989RB-I00) (2020-2022)**

Chronic low-grade inflammation, a distinctive feature of obesity, is directly related to the development of a number of diseases that are commonly diagnosed to obese individuals, including, but not limited to, type 2 diabetes, cardiovascular disease, and some types of cancer. In obesity, stressed adipocytes release high amounts of saturated fatty acids, due to dysregulated lipolysis. Macrophages present in the tissue are activated by the fatty acid overload, producing proinflammatory cytokines, which perpetuates an inflammatory state. It is now recognized that inflammasomes, i.e. intracellular multiprotein machineries that generate mature interleukin-1β, play a fundamental role in the onset of obesity-associated type-2 diabetes and cardiovascular disease. However, the molecular mechanisms that control inflammasome activation in such a context still remain to be elucidated. We have previously found that a key enzyme in lipid metabolism, the phosphatidic acid phosphatase lipin-2, restricts the classical activation of the NLRP3 inflammasome in macrophages. In preliminary experiments we have noted that lipin-2 also restricts inflammasome activation during saturated fatty acid overload, suggesting that this enzyme may function as a cellular brake that reduces the deleterious effects of cellular stressors which activate the inflammasome. The present research proposal is articulated around four specific objectives, all of them exploring completely uncharted territory, that should provide key information to understand how dysregulated lipid metabolism and the appearance of new lipid signatures activates the NLRP3 inflammasome, and the antagonistic role played by lipin-2. (i) to analyze by mass spectrometry-based lipidomic approaches lipid species that change as a consequence of metabolic activation of macrophages; (ii) to identify and characterize the enzymatic pathways involved in the generation of those lipids and assess their impact on inflammasome activation; (iii) to synthesize potentially interesting lipids as a tool to unveil their impact on interleukin-1β production; and (iv) to evaluate the involvement of specific molecular species of the lipids identified in previous aims in animal models of inflammasome activation and obesity. Completion of this proposal will lay a solid foundation for understanding, at a molecular level, new concepts on lipid metabolism during inflammation, and on lipin-2-mediated mechanisms to oppose inflammation that may open the door to future therapeutic manipulation.

Publications derived from this grant (69).

(15) **Regulation of inflammasome activity by anti-inflammatory lipids (Regional Government of Castile and León, Department of Education, ref. CSI141P20) (2021-2023)**

The inflammasome is an intracellular multiprotein

complex responsible for generating IL-1β, which participates in the destruction of pathogenic microorganisms. It is activated by pathogen-associated molecular patterns and also by endogenous molecules generated by the body during situations of risk or damage. Because of the latter, inflammasome activation is also central to the development of non-microbial diseases such as type 2 diabetes, rheumatoid arthritis and atherosclerosis. Therefore, it is important to understand the mechanisms that antagonize inflammasome activation, as a strategy to cure or alleviate the aforementioned diseases. Recently, it has been described that omega-3 fatty acids can inhibit inflammasome activation, although the molecular mechanisms involved have not been well defined. Preliminary experiments in our laboratory suggest that the incorporation of these fatty acids into cell lipids is a necessary step for the inhibitory omega-3 effect on IL-1β generation to be observed. Also, we have found that a lipid metabolic enzyme called lipin-2, exerts inhibitory effects on inflammasome activation. As a matter of fact, mutations of this enzyme produce in humans an autoinflammatory disease (Majeed syndrome) whose symptoms are alleviated by anti-IL-1β therapy. Lipin-2 possesses phosphatidic acid phosphohydrolase activity and could, therefore, participate in the generation of potential inhibitor lipids of the inflammasome. Based on these observations, the general objective of this project is to define novel routes for the involvement of omega-3 fatty acids in the inhibition of inflammasome activation via generation of lipids containing omega-3 fatty acyl esters, as well as to establish the molecular mechanisms by which lipin-2 dampens inflammasome activation, and verify whether they involve generation of these novel omega-3 lipid molecules. The proposal is divided into four specific objectives which are defined as follows: (i) to establish the omega-3 lipidome of human monocytes and macrophages, (ii) to isolate and/or chemically synthesize/derivatize potentially interesting omega-3 lipid esters and deliver them into cells, (iii) to explore the biological role of specific omega-3-contining lipid esters within anti-inflammatory signaling pathways, and (iv) to define novel anti-inflammatory routes regulated by lipin-2, potentially involving omega-3 lipid esters. Overall we will generate new data to better understand the biology of the inflammasome and its implications in pathophysiology. Our work will define new omega-3-derived molecules and pathways involved in the inhibition of the inflammasome, the manipulation of which could be used for the development of therapeutic strategies for the treatment of inflammatory conditions where the inflammasome plays a central role.

No publications derived from this grant yet.

REFERENCES

1. Balsinde, J. 2002. Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J*. 364: 695–702.
2. Balboa, M. A., and J. Balsinde. 2002. Involvement of calcium-independent phospholipase A_2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem*. 277: 40384–40389.
3. Balsinde, J., M. V. Winstead, and E. A. Dennis. 2002. Phospholipase A_2 regulation of arachidonic acid mobilization. *FEBS Lett.* 531: 2–6.
4. Fuentes, L., R. Pérez, M.L. Nieto, J. Balsinde, and M.A. Balboa. 2003. Bromoenol lactone promotes cell death by a mechanism involving phosphatidate phosphohydrolase-1 rather than calcium-independent phospholipase A_2. *J. Biol. Chem.* 278: 44683–44690.
5. Balboa, M. A., R. Pérez, and J. Balsinde. 2003. 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: 989–994.
6. Balboa, M. A., Y. Sáez, and J. Balsinde. 2003. Calcium-independent phospholipase A_2 is required for lysozyme secretion in U937 promonocytes. *J. Immunol*. 170: 5276–5280.
7. Pérez, R., R. Melero, M.A. Balboa, and J. Balsinde. 2004. 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: 40385-40391.
8. Balsinde, J., and M.A. Balboa. 2005. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A_2 in activated cells. *Cell. Signal*. 17: 1052-1062.
9. Casas, J., M.A. Gijón, A.G. Vigo, M.S. Crespo, J. Balsinde, and M.A. Balboa. 2006. 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: 155-162.
10. Casas, J., M.A. Gijón, A.G. Vigo, M.S. Crespo, J. Balsinde, and M.A. Balboa. 2006. Overexpression of cytosolic group IVA phospholipase A_2 protects cells from calcium-dependent death. *J. Biol. Chem*. 281: 6106–6116.
11. Pérez, R., X. Matabosch, A. Llebaria, M.A. Balboa, and J. Balsinde. 2006. Blockade of arachidonic acid incorporation into phospholipids induces apoptosis in U937 promonocytic cells. *J. Lipid Res*. 47: 484–491.
12. Shirai, Y., J. Balsinde, and E. A. Dennis. 2005. 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: 119–129.
13. Pérez, R., M. A. Balboa, and J. Balsinde. 2006. Involvement of group VIA calcium-independent phospholipase A_2 in macrophage engulfment of hydrogen peroxide-treated U937 cells. *J. Immunol.* 176: 2555-2561.
14. Balboa, M. A., and J. Balsinde. 2006. Oxidative stress and arachidonic acid mobilization. *Biochim. Biophys. Acta* 1761: 385–391.
15. Balsinde, J., R. Pérez, and M.A. Balboa. 2006. Calcium-independent phospholipase A_2 and apoptosis, *Biochim. Biophys. Acta* 1761: 1344–1350.
16. Ruipérez, V., J. Casas, M. A. Balboa, and J. Balsinde. 2007. Group V phospholipase A_2-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages. *J. Immunol*. 179: 631–638.
17. Pindado, J., J. Balsinde, and M. A. Balboa. 2007. TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase 2/cyclooxygenase-2 pathway. *J. Immunol.* 179: 4821–4828.
18. Balboa, M. A., R. Pérez, and J. Balsinde. 2008. Calcium-independent phospholipase A_2 mediates proliferation of human promonocytic U937 cells. *FEBS J*. 275: 1915–1924.
19. Balgoma, D., O. Montero, M. A. Balboa, and J. Balsinde. 2008. Calcium-independent phospholipase A_2-mediated formation of 1,2-diarachidonoylglycerophosphoinositol in monocytes. *FEBS J*. 275: 6180–6191.
20. Gubern, A., J. Casas, M. Barceló, D. Barneda, X. de la Rosa, R. Masgrau, F. Picatoste, J. Balsinde, M. A. Balboa, and E. Claro. 2008. Group IVA phospholipase A_2 is necessary for the biogenesis of lipid droplets. *J. Biol. Chem.* 283: 27369–27382.
21. Gubern, A., M. Barceló, J. Casas, D. Barneda, R. Masgrau, F. Picatoste, J. Balsinde, M. A.Balboa, and E. Claro. 2009. Lipid droplet biogenesis induced by stress involves triacylglycerol synthesis that depends on group VIA phospholipase A_2. *J. Biol. Chem.* 284: 5697–5708.
22. Ruipérez, V., A. M. Astudillo, M. A. Balboa, and J. Balsinde. 2009. Coordinate regulation of TLR-mediated arachidonic acid mobilization in macrophages by group IVA and group V phospholipase A_2s. *J. Immunol*. 182: 3877–3883.

23. Casas, J., C. Meana, E. Esquinas, M. Valdearcos, J. Pindado, J. Balsinde, and M. A. Balboa. 2009. Requirement of JNK-mediated phosphorylation for translocation of group IVA phospholipase A_2 to phagosomes in human macrophages. *J. Immunol*. 183: 2767–2774.
24. Pérez-Chacón, G., A. M. Astudillo, D. Balgoma, M. A. Balboa, and J. Balsinde. 2009. Control of free arachidonic acid levels by phospholipases A_2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103–1113.
25. Gubern, A., M. Barceló, D. Barneda, J. M. López, R. Masgrau, F. Picatoste, C. E. Chalfant, J. Balsinde, M. A. Balboa, and E. Claro. 2009. JNK and ceramide kinase govern the biogenesis of lipid droplets through activation of group IVA phospholipase A_2. *J. Biol. Chem.* 284: 32359–32369.
26. Pérez-Chacón, G., A. M. Astudillo, V. Ruipérez, M. A. Balboa, and J. Balsinde. 2010. Signaling role for lyso-phosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. *J. Immunol*. 184: 1071–1078.
27. Casas, J., M. Valdearcos, J. Pindado, J. Balsinde, and M. A. Balboa. 2010. The cationic cluster of group IVA phospholipase A_2 (Lys488/Lys541/Lys543/Lys544) is involved in translocation of the enzyme to phagosomes in human macrophages. *J. Lipid Res*. 51: 388–399.
28. Balgoma, D., A. M. Astudillo, G. Pérez-Chacón, O. Montero, M. A. Balboa, and J. Balsinde. 2010. Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids. *J. Immunol.* 184: 3857–3865.
29. Balgoma, D., O. Montero, M. A. Balboa, and J. Balsinde. 2010. Lipidomic approaches to the study of phospholipase A_2-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 92: 645–650.
30. Astudillo, A. M., G. Pérez-Chacón, D. Balgoma, L. Gil-de-Gómez, V. Ruipérez, C. Guijas, M. A. Balboa, and J. Balsinde. 2011. Influence of cellular arachidonic acid levels on phospholipid remodeling and CoA-independent transacylase activity in human monocytes and U937 cells. *Biochim. Biophys. Acta* 1811: 97–103.
31. Valdearcos, M., E. Esquinas, C. Meana, L. Gil-de-Gómez, C. Guijas, J. Balsinde, and M. A. Balboa. 2011. Subcellular localization and role of lipin-1 in human macrophages. *J. Immunol*. 186: 6004–6013.
32. Barroso, G., R. Rodríguez-Calvo, L. Serrano-Marco, A. M. Astudillo, J. Balsinde, X. Palomer, and M. Vázquez-Carrera. 2011. The PPARβ/δ activator GW501516 prevents the down-regulation of AMPK caused by a high-fat diet in liver and amplifies the PGC-1α-lipin 1-PPARα pathway leading to increased fatty acid oxidation. *Endocrinology* 152: 1848–1859.
33. Astudillo, A. M., G. Pérez-Chacón, C. Meana, D. Balgoma, A. Pol, M. A. Del Pozo, M. A. Balboa, and J. Balsinde. 2011. Altered arachidonate distribution in macrophages from caveolin-1 null mice leading to reduced eicosanoid synthesis. *J. Biol. Chem*. 286: 35299–35307.
34. Bosch, M., M. Marí, A. Herms, A. Fernández, A. Fajardo, A. Kassan, A. Giralt, A. Colell, D. Balgoma, E. Barbero, E. González-Moreno, N. Matías, F. Tebar, J. Balsinde, M. Camps, C. Enrich, S. P. Gross, C. García-Ruiz, E. Pérez-Navarro, J. C. Fernández-Checa, and A. Pol. 2011. Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility. *Curr. Biol.* 21: 681–686.
35. Astudillo, A. M., D. Balgoma, M. A. Balboa, and J. Balsinde. 2012. Dynamics of arachidonic acid mobilization by inflammatory cells. *Biochim. Biophys. Acta* 1821: 249–256.
36. Valdearcos, M., E. Esquinas, C. Meana, L. Peña, L. Gil-de-Gómez, J. Balsinde., and M. A. Balboa. 2012. Lipin-2 reduces proinflammatory signaling induced by saturated fatty acids in macrophages. *J. Biol. Chem.* 287: 10894–10904.
37. Guijas, C., A. M. Astudillo, L. Gil-de-Gómez, J. M. Rubio, M. A. Balboa, and J. Balsinde. 2012. Phospholipid sources for adrenic acid mobilization in RAW 264.7 macrophages: comparison with arachidonic acid. *Biochim. Biophys. Acta* 1821: 1386–1393.
38. Guijas, C., G. Pérez-Chacón, A. M. Astudillo, J. M. Rubio, L. Gil-de-Gómez, M. A. Balboa, and J. Balsinde. 2012. Simultaneous activation of p38 and JNK by arachidonic acid stimulates the cytosolic phospholipase A_2-dependent synthesis of lipid droplets in human monocytes. *J. Lipid Res*. 53: 2343–2354.
39. Gil-de-Gómez, L., A. M. Astudillo, C. Meana, J. M. Rubio, C. Guijas, M. A. Balboa, and J. Balsinde. 2013. A phosphatidylinositol species acutely generated by activated macrophages regulates innate immune responses. *J. Immunol*. 190: 5169–5177.
40. Fernández, A., N. Matías, R. Fucho, V. Ribas, C. V. Montfort, N. Nuño, A. Baulies, L. Martínez, N. Tarrats, M. Mari, A. Colell, A. Morales, L. Dubuquoy, P. Mathurin, R. Bataller, J. Caballería, M. Elena, J. Balsinde, N. Kaplowitz, C. García-Ruiz, and J. C. Fernández-Checa. 2013. Acid sphingomyelinase is required for chronic alcohol-induced hepatic endoplasmic reticulum stress and mitochondrial cholesterol loading. *J. Hepatol.* 59: 805–813.
41. Gil-de-Gómez, L., A. M. Astudillo, C. Guijas, V. Magrioti, G. Kokotos, M. A. Balboa, and J. Balsinde. 2014. Cytosolic group IVA and calcium-independent group VIA phospholipase A_2s act on distinct phospholipid pools in zymosan-stimulated mouse peritoneal macrophages. *J. Immunol*. 192: 752–762.

42. Castaño, C., E. Larequi, I. Belza, A. M. Astudillo, E. Martínez-Ansó, J. Balsinde, J. Argemi, T. Aragón, M. J. Moreno-Aliaga, J. Muntane, J. Prieto, and M. Bustos. 2014. Cardiotrophin-1 eliminates hepatic steatosis in obese mice by mechanisms involving AMPK activation. *J. Hepatol.* 60: 1017–1025.
43. Leiguez, E., K. C. Giannotti, V. Moreira, M. H. Matsubara, J. M. Gutiérrez, B. Lomonte, J. P. Rodríguez, J. Balsinde, and C. Teixeira. 2014. Critical role of TLR2 and MyD88 for functional response of macrophages to a group IIA secreted phospholipase A_2 from snake venom. *PLoS One* 9: e93741.
44. Fucho, R., L. Martínez, A. Baulies, N. Tarrats, A. Fernández, V. Ribas, A. M. Astudillo, J. Balsinde, P. García-Roves, M. Elena, I. Bergheim, S. Lotersztajn, C. Trautwein, H. Appelqvist, A. W. Paton, J. C. Paton, M. J. Czaja, N. Kaplowitz, J. C. Fernández-Checa, and C. García-Ruiz. 2014. Acid sphingomyelinase regulates autophagy and lysosomal membrane permeabilization and its inhibition prevents early stage nonalcoholic steatohepatitis. *J. Hepatol.* 61: 1126–1134.
45. Meana, C., L. Peña, G. Lordén, E. Esquinas, C. Guijas, M. Valdearcos, J. Balsinde., and M. A. Balboa. 2014. Lipin-1 integrates lipid synthesis with proinflammatory responses during TLR activation in macrophages. *J. Immunol.* 193: 4614–4622.
46. Guijas, C., J. P. Rodríguez, J. M. Rubio, M. A. Balboa, and J. Balsinde. 2014. Phospholipase A_2 regulation of lipid droplet formation. *Biochim. Biophys. Acta* 1841: 1661–1671.
47. Rubio, J. M., J. P. Rodríguez, L. Gil-de-Gómez, C. Guijas, M. A. Balboa, and J. Balsinde. 2015. Group V secreted phospholipase A_2 is up-regulated by interleukin-4 in human macrophages and mediates phagocytosis via hydrolysis of ethanolamine phospholipids. *J. Immunol.* 194: 3327–3339.
48. Pardo, V., A. González-Rodríguez, C. Guijas, J. Balsinde, and A. M. Valverde. 2015. Opposite cross-talk by oleate and palmitate on insulin signaling in hepatocytes through macrophage activation. *J. Biol. Chem.* 290: 11663–11677.
49. Santos-Nogueira, E., C. López-Serrano, J. Hernández, N. Lago, A. M. Astudillo, J. Balsinde, G. Estivill-Torrús, F. R. de Fonseca, J. Chun, and R. López-Vales. 2015. Activation of lysophosphatidic acid receptor type 1 (LPA1) contributes to pathophysiology of spinal cord injury. *J. Neurosci.* 35: 10224–10235.
50. Guijas, C., C. Meana, A. M. Astudillo, M. A. Balboa, and J. Balsinde. 2016. Foamy monocytes are enriched in cis-7-hexadecenoic fatty acid (16:1n-9), a possible biomarker for early detection of cardiovascular disease. *Cell Chem. Biol*. 23: 689–699.
51. Sala-Vila, A., I. Navarro-Lérida, M. Sánchez-Alvarez, M. Bosch, C. Calvo, J.A. López, E. Calvo, C. Ferguson, M. Giacomello, A. Serafini, L. Scorrano, J. Enríquez, J. Balsinde, R. Parton, J. Vázquez, A. Pol, and M.A. del Pozo. 2016. Interplay between hepatic mitochondria-associated membranes, lipid metabolism and caveolin-1 in mice. *Sci. Rep*. 6: 27351.
52. Peña, L., C. Meana, A. M. Astudillo, G. Lordén, M. Valdearcos, H. Sato, M. Murakami, J. Balsinde, and M. A. Balboa. 2016. Critical role for cytosolic group IVA phospholipase A_2 in early adipocyte differentiation and obesity. *Biochim. Biophys. Acta* 1861: 1083–1095.
53. Lordén, G., I. Sanjuán-García, N. de Pablo, C. Meana, I. Alvarez-Miguel, M. T. Pérez-García, P. Pelegrín, J. Balsinde, and M. A. Balboa. 2017. Lipin-2 regulates NLRP3 inflammasome by affecting P2X7 receptor activation. *J. Exp. Med.* 214: 511–528.
54. Meana, C., G.G. Rostán, L. Peña, L., G. Lordén, A. Cubero, A. Orduña, B. Györffy, J. Balsinde, and M.A. Balboa. 2018. The phosphatidic acid phosphatase lipin-1 facilitates inflammation-driven colon carcinogenesis. *JCI Insight* 3: e97506.
55. Balboa, M.A., N. de Pablo, C. Meana, and J. Balsinde. 2019. The role of lipins in innate immunity and inflammation. *Biochim. Biophys. Acta* 1864: 1328–1337.
56. Albacete, L.A., I. Navarro-Lérida, J.A. López, I. Martín-Padura, A.M. Astudillo, A. Ferrarini, M. Van-der-Heyden, J. Balsinde, G. Orend, J. Vázquez, and M.A. del Pozo. 2020. Extracellular matrix deposition is driven by caveolin1-dependent regulation of exosomal biogenesis and cargo sorting. *J. Cell Biol*. 219: e202006178.
57. Gil-de-Gómez, L., A.M. Astudillo, P. Lebrero, M.A. Balboa, and J. Balsinde. 2017. Essential role for ethanolamine plasmalogen hydrolysis in bacterial lipopolysaccharide priming of macrophages for enhanced arachidonic acid release. *Front. Immunol*. 8: 1251.
58. Astudillo, A.M., C. Meana, C. Guijas, L. Pereira, P. Lebrero, M.A. Balboa, and J. Balsinde. 2018. Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. *J. Lipid Res*. 59: 237–249.
59. Rubio, J. M., A. M. Astudillo, J. Casas, M. A. Balboa, and J. Balsinde. 2018. Regulation of phagocytosis in macrophages by membrane ethanolamine plasmalogens. *Front. Immunol*. 9: 1723.
60. Astudillo, A.M., M.A. Balboa, and J. Balsinde. 2019. Selectivity of phospholipid hydrolysis by phospholipase A_2 enzymes in activated cells leading to polyunsaturated fatty acid mobilization. *Biochim. Biophys. Acta* 1864: 772–783.
61. Vázquez, P., C. Hernández-Sánchez, C. Escalona-Garrido, L. Pereira, C. Contreras, M. López, J. Balsinde, F. de Pablo, and A.M. Valverde. 2018. Increased FGF21 in brown adipose tissue of tyrosine hydroxylase heterozygous mice:

implications for cold adaptation. *J. Lipid Res*. 59: 2308–2320.
62. Rodríguez, J. P., C. Guijas, A. M. Astudillo, J. M. Rubio, M. A. Balboa, and J. Balsinde. 2019. Sequestration of 9-hydroxystearic acid in FAHFA (fatty acid esters of hydroxy fatty acids) as a protective mechanism for colon carcinoma cells to avoid apoptotic cell death. *Cancers* 11: 524.
63. Lebrero, P., A.M. Astudillo, J.M. Rubio, L. Fernández-Caballero, G. Kokotos, M.A. Balboa, and J. Balsinde. 2019. Cellular plasmalogen content does not influence arachidonic acid levels or distribution in macrophages: a role for cytosolic phospholipase $A_2\gamma$ in phospholipid remodeling. *Cells* 8: 799.
64. Guijas, C., M.A. Bermúdez, C. Meana, A.M. Astudillo, L. Pereira, L. Fernández-Caballero, M.A. Balboa, and J. Balsinde. 2019. Neutral lipids are not a source of arachidonic acid for lipid mediator signaling in human foamy monocytes. *Cells* 8: 941.
65. Gutiérrez-Herrero, S., C. Fernández-Infante, L. Hernández-Cano, S. Ortiz-Rivero, C. Guijas, V. Martín-Granado, J.R. González-Porras, J. Balsinde, A. Porras, and C. Guerrero. 2020. C3G contributes to platelet activation and aggregation by regulating major signaling pathways. *Signal Transduct. Target. Ther*. 5: 29.
66. Monge, P., A. Garrido, J.M. Rubio, V. Magrioti, G. Kokotos, M.A. Balboa, and J. Balsinde, J. 2020. The contribution of cytosolic group IVA and calcium-independent group VIA phospholipase A_2s to adrenic acid mobilization in murine macrophages. *Biomolecules* 10: 542.
67. Rodríguez, J.P., E. Leiguez, C. Guijas, B. Lomonte, J.M. Gutiérrez, C. Teixeira, M.A. Balboa, and J. Balsinde. 2020. A lipidomic perspective of the action of group IIA secreted phospholipase A_2 on human monocytes: lipid droplet biogenesis and activation of cytosolic phospholipase $A_2\alpha$. *Biomolecules* 10: 891.
68. Gil-de-Gómez, L., P. Monge, J.P. Rodríguez, A.M. Astudillo, M.A. Balboa, and J. Balsinde. 2020. Phospholipid arachidonic acid remodeling during phagocytosis in mouse peritoneal macrophages. *Biomedicines* 8: 274.
69. Astudillo, A.M., C. Meana, M.A. Bermúdez, A. Pérez-Encabo, M.A. Balboa, and J. Balsinde. 2020. Release of anti-Inflammatory palmitoleic acid and its positional isomers by mouse peritoneal macrophages. *Biomedicines* 8: 480.

THE EICOSANOID RESEARCH DIVISION
VALLADOLID