

The Eicosanoid Research Division @ IBGM

-- Research Support History --

Current Support

ERD-12

Regulation of the Inflammatory Process by Lipin: Signaling Role in Human Macrophage Signaling

Agency – Spanish Ministry of Science and Education

Reference – SAF2010-18831

Period – Jan 2011 - Dec 2013

Amount – 254,100 €

Principal Investigator – M. Balboa

Number of Investigators Involved – 5

Summary - Macrophages are key cells that help in the management of inflammation, but they can also contribute to exacerbating it by generating excessive pro-inflammatory molecules. The study of the signaling programs that operate during macrophage activation could give us a clue to manipulate it, and could help us generate new drugs and treatments for the cure and prevention of chronic inflammatory diseases. The overall goal of this project is to define the role of lipin, a phosphatidate phosphatase, in the regulation of macrophage biology. Lipin can act as a signaling molecule and as a fat-storage mediator. We propose to study the mechanism by which lipin changes the activation state of macrophages and its impact in animal models of inflammation. The specific aims are: 1) to define which and how the metabolites that are generated and cleared by lipin after macrophage stimulation influence the generation of pro-inflammatory molecules and their repercussion on pulmonary and peritoneal inflammation models, 2) to define the role of lipin during macrophage activation by fatty acids and their impact in high fat diet-induced inflammation, 3) to define the protein domains and mechanisms by which lipin attaches and/or translocates to subcellular compartments, and 4) to define the role of lipin in the formation and maintenance of lipid droplets. Completion of this proposal may open new avenues for the control of excessive macrophage activation and hence, for the future management of inflammatory-related illnesses.

Publications Derived from This Grant

Astudillo, A. M., Pérez-Chacón, G., Balgoma, D., Gil de Gómez, L., Ruipérez, V., Guijas, C., Balboa, M. A. & Balsinde, J. (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.

Valdearcos, M., Esquinas, E., Meana, C., Gil de Gómez, L., Guijas, C., Balsinde, J. & Balboa, M. A (2011) Subcellular localization and role of lipin-1 in human macrophages. *J. Immunol.* (in press).

ERD-11

Mass Spectrometry Lipidomic Profiling of Human Macrophage Polarization

Agency – Spanish Ministry of Science and Education

Reference – BFU2010-18826

Period – Jan 2011 - Dec 2013

Amount - € 266,200

Principal Investigator – J. Balsinde

Number of Investigators Involved – 5

Summary - 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 macrophage. With this information, molecular “fingerprints” of each macrophage state will be obtained that permit 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

Astudillo, A. M., Pérez-Chacón, G., Balgoma, D., Gil de Gómez, L., Ruipérez, V., Guijas, C., Balboa, M. A. & Balsinde, J. (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.

Barroso, G., Rodríguez-Calvo, R., Serrano-Marco, L., Astudillo, A. M., Balsinde, J., Palomer, X. & Vázquez-Carrera, M. (2011) The PPAR β/δ activator GW501516 prevents the downregulation of AMPK caused by a high-fat diet in liver and amplifies the PGC-1 α -lipin 1-PPAR α pathway leading to increased fatty acid oxidation. *Endocrinology* (in press).

Valdearcos, M., Esquinas, E., Meana, C., Gil de Gómez, L., Guijas, C., Balsinde, J. & Balboa, M. A (2011) Subcellular localization and role of lipin-1 in human macrophages. *J. Immunol.* (in press).

ERD-10

Regulation of Inflammation by Lipin, an Enzyme Involved in Obesity

Agency – Regional Government of Castile and Leon

Reference – BIO39/VA04/10

Period – Jan 2010 - Dec 2011

Amount - € 28,858

Principal Investigator – M. Balboa

Number of Investigators Involved – 6

Summary - During inflammation, different cellular types move to the inflammatory foci to help in the tasks of repairing and cleaning. Monocytes/macrophages are one of these. Although the scientific community has dedicated much effort to the study of these immune cells, a major part of the molecular mechanisms that govern their activation/deactivation actions during inflammation remain unanswered. The present project focuses on the role of a key enzyme in lipid metabolism, the phosphatidic acid phosphatase (PAP), in activation and survival of the macrophages. PAP has recently been sequenced and identified to be the same as lipin, an enzyme whose overexpression produces obesity, and its absence produces loss of adipose tissue. Obesity is now known to induce a chronic activation of the innate immune system, and the macrophages are the cells chiefly implied in the production of proinflammatory cytokines under these settings. Study on the biological role of PAP/Lipin could unveil new connections between obesity and inflammation.

Publications Derived from This Grant

None yet.

The Eicosanoid Research Division @ IBGM

-- Research Support History --

Past Support

ERD-09

Role of Calcium-independent Phospholipase A₂ in Oxidative Stress and Apoptosis

Agency - Regional Government of Castile and León

Reference – CSI09A08

Period - Jan 2008 - Dec 2010

Amount - € 12,200

Principal Investigator – J. Balsinde

Number of Investigators Involved – 8

Summary - 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 A₂ activity is augmented during oxidative stress and the apoptotic processes that ordinarily ensue. Previous work by our group has shown that calcium-independent phospholipase A₂ (iPLA₂) 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 iPLA₂; (ii) the molecular mechanisms associated to hydrogen peroxide-induced apoptosis; (iii) changes in iPLA₂ activity and/or physical state that may account for its enhanced capacity to destroy membrane phospholipid; (iv) the role of iPLA₂-derived products, free fatty acids and lysophospholipids, during oxidant-induced apoptosis; and (v) the role of iPLA₂ on the phagocytosis of apoptotic cells by phagocytes.

Publications Derived from This Grant (8)

Casas, J., Meana, C., Esquinas, E., Valdearcos, M., Pindado, J., Balsinde, J. & Balboa, M. A. (2009) Requirement of JNK-mediated phosphorylation for translocation of group IVA phospholipase A₂ to phagosomes in human macrophages. *J. Immunol.* 183: 2767-2774.

Pérez-Chacón, G., Astudillo, A. M., Balgoma, D., Balboa, M. A. & Balsinde, J. (2009) Control of free arachidonic acid levels by phospholipases A₂ and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103-1113.

Pérez-Chacón, G., Astudillo, A. M., Ruipérez, V., Balboa, M. A. & Balsinde, J. (2010) Signaling role for lysophosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. *J. Immunol.* 184:1071-1078.

Casas, J., Valdearcos, M., Pindado, J., Balsinde, J. & Balboa, M. A. (2010) The cationic cluster of group IVA phospholipase A₂ (Lys488/Lys541/Lys543/Lys544) is involved in translocation of the enzyme to phagosomes in human macrophages. *J. Lipid Res.* 51: 388-399.

Balgoma, D., Astudillo, A. M., Pérez-Chacón, G., Montero, O., Balboa, M. A. & Balsinde, J. (2010) Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids. *J. Immunol.* 184: 3857-3865.

Balgoma, D., Montero, O., Balboa, M. A. & Balsinde, J. (2010) Lipidomic approaches to the study of phospholipase A₂-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 92: 645-650.

Astudillo, A. M., Pérez-Chacón, G., Balgoma, D., Gil de Gómez, L., Ruipérez, V., Guijas, C., Balboa, M. A. & Balsinde, J. (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.

Valdearcos, M., Esquinas, E., Meana, C., Gil de Gómez, L., Guijas, C., Balsinde, J. & Balboa, M. A. (2011) Subcellular localization and role of lipin-1 in human macrophages. *J. Immunol.* (in press).

ERD-08

A Lipidomics Approach to the Study of the Innate Immune Response: Mechanisms Governing Arachidonic Acid Availability and Metabolism in Macrophages

Agency - Spanish Ministry of Science and Technology

Reference - BFU2007-67154/BMC

Period - Dec 2007 - Dec 2010

Amount - € 375,100

Principal Investigator – J. Balsinde

Number of Investigators Involved – 7

Summary - Inflammatory diseases bear strong social repercussions because of the elevated number of individuals suffering from them and the high costs of health 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 diffculted 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 (13)

Balboa, M. A., Pérez, R. & Balsinde, J. (2008) Calcium-independent phospholipase A₂ mediates proliferation of human promonocytic U937 cells. *FEBS J.* 275: 1915-1924.

Balgoma, D., Montero, O., Balboa, M. A. & Balsinde, J. (2008) Calcium-independent phospholipase A₂-mediated formation of 1,2-diarachidonoyl-glycerophosphoinositol in human monocytes. *FEBS J.* 275: 6180-6191.

Gubern, A., Barceló, M., Casas, J., Barneda, D., Masgrau, R., Picatoste, F., Balsinde, J., Balboa, M. A., & Claro, E. (2009) Lipid droplet biogenesis induced by stress involves triacylglycerol synthesis that depends on group VIA phospholipase A₂. *J. Biol. Chem.* 284: 5697-5708.

Ruipérez, V., Astudillo, A. M., Balboa, M. A. & Balsinde, J. (2009) Coordinate regulation of Toll-like receptor-mediated arachidonic acid mobilization in macrophages by group IVA and group V phospholipase A₂s. *J. Immunol.* 182: 3877-3883.

Casas, J., Meana, C., Esquinas, E., Valdearcos, M., Pindado, J., Balsinde, J. & Balboa, M. A. (2009) Requirement of JNK-mediated phosphorylation for translocation of group IVA phospholipase A₂ to phagosomes in human macrophages. *J. Immunol.* 183: 2767-2774.

Pérez-Chacón, G., Astudillo, A. M., Balgoma, D., Balboa, M. A. & Balsinde, J. (2009) Control of free arachidonic acid levels by phospholipases A₂ and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103-1113.

Gubern, A., Barceló, M., Barneda, D., López, J. M., Masgrau, R., Picatoste, F., Chalfant, C. E., Balsinde, J., Balboa, M. A. & Claro, E. (2009) JNK and ceramide kinase govern the biogenesis of lipid droplets through activation of group IVA phospholipase A₂. *J. Biol. Chem.* 284: 32359–32369.

Pérez-Chacón, G., Astudillo, A. M., Ruipérez, V., Balboa, M. A. & Balsinde, J. (2010) Signaling role for lysophosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. *J. Immunol.* 184:1071-1078.

Casas, J., Valdearcos, M., Pindado, J., Balsinde, J. & Balboa, M. A. (2010) The cationic cluster of group IVA phospholipase A₂ (Lys488/Lys541/Lys543/Lys544) is involved in translocation of the enzyme to phagosomes in human macrophages. *J. Lipid Res.* 51: 388-399.

Balgoma, D., Astudillo, A. M., Pérez-Chacón, G., Montero, O., Balboa, M. A. & Balsinde, J. (2010) Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids. *J. Immunol.* 184: 3857-3865.

Balgoma, D., Montero, O., Balboa, M. A. & Balsinde, J. (2010) Lipidomic approaches to the study of phospholipase A₂-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 92: 645-650.

Astudillo, A. M., Pérez-Chacón, G., Balgoma, D., Gil de Gómez, L., Ruipérez, V., Guijas, C., Balboa, M. A. & Balsinde, J. (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.

Valdearcos, M., Esquinas, E., Meana, C., Gil de Gómez, L., Guijas, C., Balsinde, J. & Balboa, M. A. (2011) Subcellular localization and role of lipin-1 in human macrophages. *J. Immunol.* (in press).

ERD-07

Linking Inflammation to Obesity: Regulatory Roles of Magnesium-dependent Phosphatidic Acid Phosphatase in Macrophage Physiology

Agency - Spanish Ministry of Science and Technology

Reference - SAF2007-60055

Period - Dec 2007 - Dec 2010

Amount - € 246,365

Principal Investigator – M.A. Balboa

Number of Investigators Involved –

Summary - When an inflammatory focus is established, various cell types migrate to the inflamed site to begin reactions of healing and repair. Macrophages are among these cells and play prominent roles in the initiation and resolution of the inflammatory process. Although much effort has been put into understanding the basic molecular mechanisms underlying macrophage activation during innate immune reactions, there are still key aspects that remain to be elucidated, in particular many affecting the lipid metabolism. This research proposal focuses on the study of the role of phosphatidic acid phosphatase (PAP) during macrophage activation and survival. The sequence of PAP, a key enzyme in the biosynthesis of glycerophospholipids and triacylglycerol, has recently been elucidated and, interestingly, found to be identical to the protein known as lipin, a protein known to regulate obesity. Overexpression of lipin in mice leads to obesity and deletion of the gene shows marked changes in adipose tissue distribution. It is well known that obesity is a leading factor for the induction of chronic activation of the innate immune system and that macrophages may be the relevant cell type involved by virtue of its capacity to release a wide array of proinflammatory cytokines under obesity conditions. Chronic macrophage activation under these conditions is thought to ultimately lead to insulin resistance, glucose intolerance and even diabetes. Studies on PAP/lipin may help uncover novel routes of interaction between obesity and inflammation. Accordingly, we propose to study (i) the expression of PAP/lipin in human macrophages; (ii) the subcellular localization of the enzyme; (iii) activity regulation by phosphorylation; (iv) effects of PAP/lipin overexpression and silencing on general macrophage physiology, and (v) the innate immune response of mice lacking PAP/lipin and diet-induced obese mice. These studies will aid to better understand macrophage biology and to strengthen the link between obesity and inflammation. In addition, it is expected that these studies help uncover novel molecular targets with therapeutic potential for the treatment of inflammation.

Publications Derived from This Grant (13)

Balboa, M. A., Pérez, R. & Balsinde, J. (2008) Calcium-independent phospholipase A₂ mediates proliferation of human promonocytic U937 cells. *FEBS J.* 275: 1915-1924.

Gubern, A., Casas, J., Barceló, M., Barneda, D., de la Rosa, X., Masgrau, R., Picatoste, F., Balsinde, J., Balboa, M. A., & Claro, E. (2008) Group IVA phospholipase A₂ is necessary for the biogenesis of lipid droplets. *J. Biol. Chem.* 283: 27369-27382.

Balgoma, D., Montero, O., Balboa, M. A. & Balsinde, J. (2008) Calcium-independent phospholipase A₂-mediated formation of 1,2-diarachidonoyl-glycerophosphoinositol in human monocytes. *FEBS J.* 275: 6180-6191.

Gubern, A., Barceló, M., Casas, J., Barneda, D., Masgrau, R., Picatoste, F., Balsinde, J., Balboa, M. A., & Claro, E. (2009) Lipid droplet biogenesis induced by stress involves triacylglycerol synthesis that depends on group VIA phospholipase A₂. *J. Biol. Chem.* 284: 5697-5708.

Ruipérez, V., Astudillo, A. M., Balboa, M. A. & Balsinde, J. (2009) Coordinate regulation of Toll-like receptor-mediated arachidonic acid mobilization in macrophages by group IVA and group V phospholipase A₂s. *J. Immunol.* 182: 3877-3883.

Casas, J., Meana, C., Esquinas, E., Valdearcos, M., Pindado, J., Balsinde, J. & Balboa, M. A. (2009) Requirement of JNK-mediated phosphorylation for translocation of group IVA phospholipase A₂ to phagosomes in human macrophages. *J. Immunol.* 183: 2767-2774.

Pérez-Chacón, G., Astudillo, A. M., Balgoma, D., Balboa, M. A. & Balsinde, J. (2009) Control of free arachidonic acid levels by phospholipases A₂ and lysophospholipid acyltransferases. *Biochim. Biophys. Acta* 1791: 1103-1113.

Gubern, A., Barceló, M., Barneda, D., López, J. M., Masgrau, R., Picatoste, F., Chalfant, C. E., Balsinde, J., Balboa, M. A. & Claro, E. (2009) JNK and ceramide kinase govern the biogenesis of lipid droplets through activation of group IVA phospholipase A₂. *J. Biol. Chem.* 284: 32359-32369.

Pérez-Chacón, G., Astudillo, A. M., Ruipérez, V., Balboa, M. A. & Balsinde, J. (2010) Signaling role for lysophosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. *J. Immunol.* 184:1071-1078.

Casas, J., Valdearcos, M., Pindado, J., Balsinde, J. & Balboa, M. A. (2010) The cationic cluster of group IVA phospholipase A₂ (Lys488/Lys541/Lys543/Lys544) is involved in translocation of the enzyme to phagosomes in human macrophages. *J. Lipid Res.* 51: 388-399.

Balgoma, D., Astudillo, A. M., Pérez-Chacón, G., Montero, O., Balboa, M. A. & Balsinde, J. (2010) Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids. *J. Immunol.* 184: 3857-3865.

Balgoma, D., Montero, O., Balboa, M. A. & Balsinde, J. (2010) Lipidomic approaches to the study of phospholipase A₂-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 92: 645-650.

Astudillo, A. M., Pérez-Chacón, G., Balgoma, D., Gil de Gómez, L., Ruipérez, V., Guijas, C., Balboa, M. A. & Balsinde, J. (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.

ERD-06

Regulation of Cyclooxygenase-2 Expression and Activity in Alzheimer's Disease

Agency - Fundación La Caixa

Reference - BM05-248-0

Period - Sep 2005 - Sep 2008

Amount - € 151,000

Principal Investigator – J. Balsinde

Number of Investigators Involved – 5

Summary - 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 A₂. Disregulated phospholipase A₂ activity has been correlated with several forms of acute and chronic brain injury, including cerebral trauma, cerebral ischaemia, epilepsy, schizophrenia, and in particular, Alzheimer's Disease. The expression of both phospholipase A₂ 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 A₂ 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 (3)

Ruipérez, V., Casas, J., Balboa, M. A. & Balsinde, J. (2007) Group V phospholipase A₂-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages. *J. Immunol.* 179: 631-638.

Pindado, J., Balsinde, J. & Balboa, M. A. (2007) TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A₂/cyclooxygenase-2 pathway. *J. Immunol.* 179: 4821-4828.

Balboa, M. A., Pérez, R. & Balsinde, J. (2008) Calcium-independent phospholipase A₂ mediates proliferation of human promonocytic U937 cells. *FEBS J.* 275: 1915-1924.

ERD-05

The Role of Phospholipid-remodeling Phospholipase A₂ During Apoptosis and Oxidative Stress

Agency - Spanish Ministry of Education and Science

Reference - SAF2004-04676

Period - Dec 2004 - Dec 2007

Amount - € 156,900

Principal Investigator – M.A. Balboa

Number of Investigators Involved –

Summary - 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 A₂ activity is augmented during oxidative stress and the apoptotic processes that ordinarily ensue. Previous studies by the principal investigator have shown that calcium-independent phospholipase A₂ (iPLA₂) 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 iPLA₂; (ii) molecular mechanisms associated to hydrogen peroxide-induced apoptosis; (iii) changes in iPLA₂ activity and/or physical state that may account for its enhanced capacity to destroy membrane phospholipid; (iv) role of iPLA₂-derived products, free fatty acids and lysophospholipids, during oxidant-induced apoptosis; and (v) role of iPLA₂ on the phagocytosis of apoptotic cells by phagocytes.

Publications Derived from This Grant (10)

Balsinde, J., and Balboa, M. A. (2005) Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A₂ in activated cells. *Cell. Signal.* 17: 1052-1062.

Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. (2006) Phosphatidylinositol 4,5-bisphosphate anchors cytosolic group IVA phospholipase A₂ to perinuclear membranes and decreases its calcium requirement for translocation in live cells. *Mol. Biol. Cell* 17: 155-162.

Pérez, R., Balboa, M. A. & Balsinde, J. (2006) Involvement of group VIA calcium-independent phospholipase A₂ in macrophage engulfment of hydrogen peroxide-treated U937 cells. *J. Immunol.* 176: 2555-2561.

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

Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. (2006) Overexpression of cytosolic group IVA phospholipase A₂ protects cells from calcium-dependent death. *J. Biol. Chem.* 281: 6106-6116.

Balboa, M. A. & Balsinde, J. (2006) Oxidative stress and arachidonic acid mobilization. *Biochim. Biophys. Acta* 1761: 385-391.

Balsinde, J., Pérez, R. & Balboa, M. A. (2006) Calcium-independent phospholipase A₂ and apoptosis. *Biochim. Biophys. Acta* 1761: 1344-1350.

Ruipérez, V., Casas, J., Balboa, M. A. & Balsinde, J. (2007) Group V phospholipase A₂-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages. *J. Immunol.* 179: 631-638.

Pindado, J., Balsinde, J. & Balboa, M. A. (2007) TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A₂/cyclooxygenase-2 pathway. *J. Immunol.* 179: 4821-4828.

Balboa, M. A., Pérez, R. & Balsinde, J. (2008) Calcium-independent phospholipase A₂ mediates proliferation of human promonocytic U937 cells. *FEBS J.* 275: 1915-1924.

ERD-04

Regulation of Cyclooxygenase-2 Expression by Phospholipase A₂-derived Lipid Products

Agency - Spanish Ministry of Education and Science

Reference - BFU2004-01886/BMC

Period - Dec 2004 - Dec 2007

Amount - € 173,650

Principal Investigator – J. Balsinde

Number of Investigators Involved – 2

Summary - 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 A₂ as an enzyme responsible for generating free arachidonic acid to be used for the biosynthesis of prostaglandins. In addition, group V phospholipase A₂ 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 A₂ 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 (11)

Balsinde, J., and Balboa, M. A. (2005) Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A₂ in activated cells. *Cell. Signal.* 17: 1052-1062.

Shirai, Y., Balsinde, J. & Dennis, E. A. (2005) Localization and functional interrelationships among cytosolic group IV, secreted group V, and Ca²⁺-independent group VI phospholipase A₂s in P388D₁ macrophages using GFP/RFP constructs. *Biochim. Biophys. Acta* 1735: 119-129.

Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. (2006) Phosphatidylinositol 4,5-bisphosphate anchors cytosolic group IVA phospholipase A₂ to perinuclear membranes and decreases its calcium requirement for translocation in live cells. *Mol. Biol. Cell* 17: 155-162.

Pérez, R., Balboa, M. A. & Balsinde, J. (2006) Involvement of group VIA calcium-independent phospholipase A₂ in macrophage engulfment of hydrogen peroxide-treated U937 cells. *J. Immunol.* 176: 2555-2561.

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

Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. (2006) Overexpression of cytosolic group IVA phospholipase A₂ protects cells from calcium-dependent death. *J. Biol. Chem.* 281: 6106-6116.

Balboa, M. A. & Balsinde, J. (2006) Oxidative stress and arachidonic acid mobilization. *Biochim. Biophys. Acta* 1761: 385-391.

Balsinde, J., Pérez, R. & Balboa, M. A. (2006) Calcium-independent phospholipase A₂ and apoptosis. *Biochim. Biophys. Acta* 1761: 1344-1350.

Ruipérez, V., Casas, J., Balboa, M. A. & Balsinde, J. (2007) Group V phospholipase A₂-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages. *J. Immunol.* 179: 631-638.

Pindado, J., Balsinde, J. & Balboa, M. A. (2007) TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A₂/cyclooxygenase-2 pathway. *J. Immunol.* 179: 4821-4828.

Balboa, M. A., Pérez, R. & Balsinde, J. (2008) Calcium-independent phospholipase A₂ mediates proliferation of human promonocytic U937 cells. *FEBS J.* 275: 1915-1924

ERD-03

The Lipid Hypothesis in Schizophrenia, a Novel Therapeutic Approach. Inhibitors of Calcium-independent Phospholipase A₂: Synthesis, Enzyme Inhibition, and Signal Transduction

Agency - Fundació La Marató de TV3

Reference - 011232

Period - Jan 2002- Dec 2004

Amount - € 240,000

Principal Investigator – J. Balsinde

Number of Investigators Involved – 14

Summary - The overall goal of this research proposal is the generation of novel inhibitors of calcium-independent phospholipase A₂, 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 PPAR α 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 etiology of schizophrenia. Release of polyunsaturated fatty acids from phospholipids is mediated by phospholipase A₂ enzymes, some of which may be augmented in schizophrenic patients. Our working hypothesis, based on the very well known role of phospholipase A₂ 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 (8)

Balboa, M. A. & Balsinde, J. (2002) Involvement of calcium-independent phospholipase A₂ in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem.* 277: 40384-40389.

Balsinde, J., Winstead, M. V. & Dennis, E. A. (2002) Phospholipase A₂ regulation of arachidonic acid mobilization. *FEBS Lett.* 531: 2-6.

Fuentes, L., Pérez, R., Nieto, M. L., Balsinde, J. & Balboa, M. A. (2003) Bromoenol lactone promotes cell death by a mechanism involving phosphatidate phosphohydrolase-1 rather than calcium-independent phospholipase A₂. *J. Biol. Chem.* 278: 44683-44690.

Balboa, M. A., Pérez, R. & Balsinde, J. (2003) Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A₂ is regulated by cytosolic phospholipase A₂-derived hydroperoxyeicosatetraenoic acid. *J. Immunol.* 171: 989-994.

Balboa, M. A., Sáez, Y. & Balsinde, J. (2003) Calcium-independent phospholipase A₂ is required for lysozyme secretion in U937 promonocytes. *J. Immunol.* 170: 5276-5280.

Pérez, R., Melero, R., Balboa, M. A. & Balsinde, J. (2004) Role of group VIA calcium-independent phospholipase A₂ in arachidonic acid release, phospholipid fatty acid incorporation, and apoptosis in U937 cells responding to hydrogen peroxide. *J. Biol. Chem.* 279: 40385-40391.

Balsinde, J., and Balboa, M. A. (2005) Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A₂ in activated cells. *Cell. Signal.* 17: 1052-1062.

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

ERD-02

Signal Transduction Mechanisms Involved in the Activation of Phagocytic Cells

Agency - Regional Government of Castile and León

Reference - CSI 4/02

Period - Jan 2002- Dec 2004

Amount - € 6,450

Principal Investigator – J. Balsinde

Number of Investigators Involved – 2

Summary - 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 A₂. 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 A₂ activation.

Publications Derived from This Grant (7)

Balboa, M. A. & Balsinde, J. (2002) Involvement of calcium-independent phospholipase A₂ in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem.* 277: 40384-40389.

Balsinde, J., Winstead, M. V. & Dennis, E. A. (2002) Phospholipase A₂ regulation of arachidonic acid mobilization. *FEBS Lett.* 531: 2-6.

Fuentes, L., Pérez, R., Nieto, M. L., Balsinde, J. & Balboa, M. A. (2003) Bromoenol lactone promotes cell death by a mechanism involving phosphatidate phosphohydrolase-1 rather than calcium-independent phospholipase A₂. *J. Biol. Chem.* 278: 44683-44690.

Balboa, M. A., Pérez, R. & Balsinde, J. (2003) Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A₂ is regulated by cytosolic phospholipase A₂-derived hydroperoxyeicosatetraenoic acid. *J. Immunol.* 171: 989-994.

Balboa, M. A., Sáez, Y. & Balsinde, J. (2003) Calcium-independent phospholipase A₂ is required for lysozyme secretion in U937 promonocytes. *J. Immunol.* 170: 5276-5280.

Pérez, R., Melero, R., Balboa, M. A. & Balsinde, J. (2004) Role of group VIA calcium-independent phospholipase A₂ in arachidonic acid release, phospholipid fatty acid incorporation, and apoptosis in U937 cells responding to hydrogen peroxide. *J. Biol. Chem.* 279: 40385-40391.

Balsinde, J., and Balboa, M. A. (2005) Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A₂ in activated cells. *Cell. Signal.* 17: 1052-1062.

ERD-01

Intracellular Signaling Mechanisms Regulating Prostaglandin Biosynthesis in Immunoinflammatory Cells

Agency - Spanish Ministry of Science and Technology

Reference - BMC2001-2244

Period - Dec 2001- Dec 2004

Amount - € 105,615

Principal Investigator – J. Balsinde

Number of Investigators Involved – 3

Summary - The prostaglandins are a family of oxygenated derivatives of arachidonic acid that potentially 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 by immunoinflammatory cells. 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 A₂. 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 A₂ activation; 3) novel pathways for the regulation of prostaglandin synthesis via polyphosphoinositides.

Publications Derived from This Grant (10)

Balboa, M. A. & Balsinde, J. (2002) Involvement of calcium-independent phospholipase A₂ in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem.* 277: 40384-40389.

Balsinde, J. (2002) Roles of various phospholipases A₂ in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J.* 364: 695-702.

Balsinde, J., Winstead, M. V. & Dennis, E. A. (2002) Phospholipase A₂ regulation of arachidonic acid mobilization. *FEBS Lett.* 531: 2-6.

Fuentes, L., Pérez, R., Nieto, M. L., Balsinde, J. & Balboa, M. A. (2003) Bromoenol lactone promotes cell death by a mechanism involving phosphatidate phosphohydrolase-1 rather than calcium-independent phospholipase A₂. *J. Biol. Chem.* 278: 44683-44690.

Balboa, M. A., Pérez, R. & Balsinde, J. (2003) Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A₂ is regulated by cytosolic phospholipase A₂-derived hydroperoxyeicosatetraenoic acid. *J. Immunol.* 171: 989-994.

Balboa, M. A., Sáez, Y. & Balsinde, J. (2003) Calcium-independent phospholipase A₂ is required for lysozyme secretion in U937 promonocytes. *J. Immunol.* 170: 5276-5280.

Pérez, R., Melero, R., Balboa, M. A. & Balsinde, J. (2004) Role of group VIA calcium-independent phospholipase A₂ in arachidonic acid release, phospholipid fatty acid incorporation, and apoptosis in U937 cells responding to hydrogen peroxide. *J. Biol. Chem.* 279: 40385-40391.

Balsinde, J., and Balboa, M. A. (2005) Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A₂ in activated cells. *Cell. Signal.* 17: 1052-1062.

Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. (2006) Phosphatidylinositol 4,5-bisphosphate anchors cytosolic group IVA phospholipase A₂ to perinuclear membranes and decreases its calcium requirement for translocation in live cells. *Mol. Biol. Cell* 17: 155-162.

Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. (2006) Overexpression of cytosolic group IVA phospholipase A₂ protects cells from calcium-dependent death. *J. Biol. Chem.* 281: 6106-6116.