The Eicosanoid Research Division @ IBGM

Research Subline "Lipid Signaling in Inflammation and Obesity"

1. GENERAL INFORMATION OF THE SUBLINE (PERIOD 2003-2007)

1.1. Summary

Numerous signal transduction processes involve lipids as signaling molecules. Many of these molecules are generated by phospholipases such as phospholipase A_2, which releases fatty acids like arachidonic acid, and lysophospholipids. Each of these products is implicated in signal transduction processes, but also serves as a precursor for platelet activating factor or the eicosanoids, a large familiy of mediators that includes the prostaglandins, leukotrienes, and lipoxins. All of these compounds are implicated in a wide variety of inflammatory diseases such as rheumatoid arthritis, sepsis, intestinal bowel disease, asthma as well as playing a role in cancer and premature parturition. Lipid signaling is also key to the development of obesity and the myriad of illnesses associated to it (e.g. diabetes, cardiovascular disease, etc). Other important phospholipases include phospholipase C, which controls the production of inositol-1,4,5-trisphosphate and diacylglycerol. Phospholipase D generates phosphatidic acid which subsequently can be either metabolized by phospholipase A_2 generating lysophosphophatidic acid, a potent cellular mitogen, or by phosphatidate phosphohydrolase, yielding diacylglycerol. Sphingomyelinase, a phospholipase C type enzyme, and related enzymes of sphingolipid metabolism are implicated in apoptosis.

1.2. General Objective

Phospholipases generate numerous lipid products which control much of cellular signaling and our aim is to better understand their regulation. Although our long-term goals include all of the enzymes mentioned in the preceding paragraph, our current research focuses primarily on phospholipase A_2 and phosphatidate phosphohydrolase (phosphatidic acid-specific phospholipase C; lipin). General events that we are interested in include (i) the spatiotemporal regulation of these phospholipases in a cellular context, which we study utilizing advanced microscopy techniques, (ii) pharmacological manipulation of enzymatic activity both in intact cells and in vitro, (iii) analysis of lipid metabolite production by state-of-the-art mass spectrometry (lipidomics & metabolipidomics), and (iv) the physiological functioning of phospholipases in animal models.

2. INDICATORS

2.1. Personnel (Current)

Faculty

Balboa, María Angeles (CSIC)
Balsinde Jesús (CSIC)

López Burillo, Silvia (UVa)
Montero, Olimpio (CSIC)

Postdoctorals

Meana, Clara (CIBER-ISCIII)
Pérez, Gema (CIBER-ISCIII)

Technical Support

Duque, Montserrat (CSIC)
Sáez, Yolanda (MICINN)

Predoctoral Students

Astudillo, Alma (JCYL)
Balgoma, David (FPU/MICINN)
Casas, Javier (JCYL)
Esquinas, Esperanza - JAE/CSIC
Gil de Gómez, Luis (FPI/MICINN)
Peña, Lucía (FPI/MICINN)
Pindado, José (FPI/MICINN)
Ruipérez, Violeta (La Caixa)
Valdearcos, Martín (JCYL)

2.2. Funding

2007 **Jesús Balsinde**. Una aproximación de lipidómica al estudio de la respuesta inmune innata: mecanismos que gobiernan la disponibilidad y metabolismo oxidativo de ácido araquidónico en macrófagos. MICINN (375.100 €)

2007 **Jesús Balsinde**. Red REDIMET (46.800 €).

2007 **María Angeles Balboa**. Inflamación y obesidad: dos procesos regulados por una misma enzima, la fosfatasa de ácido fosfatídico dependiente de magnesio. MICINN (246.356 €).

2006 **Jesús Balsinde**. Effects of PM02734 on the lipid metabolism of tumor cells. PharmaMar (47.600 €).

2005 **Jesús Balsinde**. Regulación de la expresión y actividad de ciclooxigenasa-2 en la enfermedad de Alzheimer. Fundación La Caixa (151.000 €).

2004 **María Angeles Balboa**. Papel de las enzimas del metabolismo lipídico en apoptosis y procesos relacionados con el estrés oxidativo. MEC (156.900 €).

2004 **Jesús Balsinde**. Regulación de la expresión de ciclooxigenasa-2 en células inmunoinflamatorias por lípidos derivados de la activación de fosfolipasa A_2. MEC (173.650 €).

2.3. Publications

2007 Pindado, J., Balsinde, J. & Balboa, M. A. TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A_2/cyclooxygenase-2 pathway. **J. Immunol.** 179: 4821-4828.

2007 Ruipérez, V., Casas, J., Balboa, M. A. & Balsinde, J. Group V phospholipase A_2-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in lipopolysaccharide-stimulated macrophages. **J. Immunol.** 179: 631-638.

2006 Balsinde, J., Pérez, R. & Balboa, M. A. Calcium-independent phospholipase A_2 and apoptosis. **Biochim. Biophys. Acta** 1761: 1344-1350.

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

2006 Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. Overexpression of cytosolic group IVA phospholipase A_2 protects cells from calcium-dependent death. **J. Biol. Chem.** 281: 6106-6116.

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

2006 Pérez, R., Balboa, M. A. & Balsinde, J. Involvement of group VIA calcium-independent phospholipase A_2 in macrophage engulfment of hydrogen peroxide-treated U937 cells. **J. Immunol.** 176: 2555-2561.

2006 Casas, J., Gijón, M. A., Vigo, A. G., Crespo, M. S., Balsinde, J. & Balboa, M. A. 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.

2005 Shirai, Y., Balsinde, J. & Dennis, E. A. 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.

2005 Balsinde, J., and Balboa, M. A. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A_2 in activated cells. **Cell. Signal.** 17: 1052-1062.

2004 Pérez, R., Melero, R., Balboa, M. A. & Balsinde, J. 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.

2003 Fuentes, L., Pérez, R., Nieto, M. L., Balsinde, J. & Balboa, M. A. 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.

2003 Balboa, M. A., Shirai, Y., Gaietta, G., Ellisman, M. E., Balsinde, J., & Dennis, E. A. Localization of group V phospholipase A_2 in caveolin-enriched granules in activated $P388D_1$ macrophage-like cells. **J. Biol. Chem.** 278: 48059-48065.

2003 Balboa, M. A., Pérez, R. & Balsinde, J. 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.

2003 Balboa, M. A., Sáez, Y. & Balsinde, J. Calcium-independent phospholipase A_2 is required for lysozyme secretion in U937 promonocytes. **J. Immunol.** 170: 5276-5280.

2.4. Student Training (Doctoral Theses)

2006 Rebeca Pérez

2.5. Other

J.B. is a Section Editor of *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*.

The group is a member of CIBERDEM, Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas.

3. CRITICAL ANALYSIS

3.1. SWOT

Strengths

World-renowned experts in our field of study.

Multidisciplinary approach (cell biology, molecular biology, biochemistry and organic chemistry approaches are combined).

Weaknesses

Our scientific surroundings are not the appropriate. Lack of critical mass is a major drawback that impacts negatively on too many aspects of our work.

The standard of publication in our field is JBC, which does not have a particularly high impact factor (despite of it being the most cited journal in the biomedical sciences). Publishing frequently in this journal is considered a measure of excellence in lipid research. However, from the outside, the low impact factor might give the impression of insufficient quality, which is obviously and utterly wrong.

Opportunities

Our association with CIBERDEM (Diabetes and Metabolic Disease Network) provides us with excellent opportunities for translational research.

Much of our work is potentially "druggable" in nature, which may provide more opportunities for collaboration with the industry in addition to those that have already

been established.

Threats

The availability of capable personnel is always uncertain. Regrettably, improving in this area depends very little on us. The city we are in does not have education programs in any of the relevant biological & biomedical disciplines, and our institute is not well positioned at a national level.

4. RELATIONAL ANALYSIS

4.1. Groups working on similar subjects

Jonathan Arm, Boston, MA, USA
Christina Leslie, Denver, CO, USA
Wonhwa Cho, Chicago, IL, USA
Makoto Murakami, Tokyo, Japan

4.2. Collaborations

Edward Dennis, La Jolla, CA, USA
Yasuhito Shirai, Kobe, Japan
Enrique Claro, Barcelona, Spain
Martin Thurnher, Innsbruck, Austria

4.3. Groups of Reference

Edward Dennis, La Jolla, CA, USA
Robert Murphy, Aurora, CO, USA
Karen Reue, Los Angeles, CA, USA
Dennis Vance, Calgary, AL, Canada
Charles Serhan, Boston, MA, USA

4.4. Selective Advantages

Good reputation and standing at an international level.

Multidisciplinary approach, ranging from Chemistry and Biochemistry to Cellular and Molecular Biology.

5. OBJECTIVES AND STRATEGY DESIGN

5.1. General Qualitative Objectives

Our general objective is to maintain our current high standards in lipid research, which have allowed us to achieve international recognition. In addition to our work on various

aspects on molecular and cell biology of lipids, we started a few years ago studies on mass spectrometry of lipids. It is our intention for the immediate future to achieve a referent status in mass spectrometry of lipids as well.

5.2. Specific Qualitative Objectives

Research Goals

Our subline's scientific goals for the 2010-1013 period can be summarized as follows:

1) Lipidomic approaches to study the innate immune response: mechanisms governing arachidonic acid availability and metabolism in macrophages - Our goal is to apply a lipidomics approach to the study of the mechanisms governing the availability and oxidative metabolism of free arachidonic acid (AA) during activation of macrophages by stimuli of the innate immune response. Availability of free AA is a limiting step for the synthesis of eicosanoids, a family of compounds with potent pro- and anti-inflammatory actions. While the pathways of AA uptake, incorporation and remodeling in glycerolipids are well documented, the individual lipid species in which the AA is stored and released from have not been identified. This is so because of the impossibility of traditional methods for lipid separation (i.e. thin-layer chromatography, liquid chromatography) to differentiate among individual lipids within various classes and subclasses. This is now possible with the advent of electrospray mass spectrometry (ESI-MS). Application of this technology to the field of lipid biochemistry has been a major breakthrough in profiling the lipidomes of cells and tissues in physiological and pathophysiological conditions. We will conduct a lipidomics analysis of all the lipid molecular species involved in AA homeostasis, from those that act as acceptors of the fatty acid to those from which the fatty acid is liberated for subsequent eicosanoid synthesis, and including as well a full survey of AA oxygenated metabolites. An analysis like this will contribute to a better understanding of the mechanisms involved in AA homeostasis and, in turn, may help identify novel metabolic targets with therapeutic potential.

2) PAP-1/Lipin1 studies in monocytes/macrophages - We will study le level of PAP-1/Lipin1 expression in basal conditions both in human monocytes and macrophages. We will also search for those conditions, proinflammatory or metabolic, that induce (or decrease) its expression in cells, trying to elucidate the intracellular signaling pathways and the transcription factors implicated in it. Due to the fact that PAP-1/Lipin1 uses lipids as substrate it seems logical to speculate (and biochemical experiments suggest it so) that the enzyme would move from the cytosol to those intracellular membranes where the lipid substrates are in. By using EGFP fused proteins and specific antibodies we will try to identify the subcellular location of this enzyme in human phagocytes, and also study the cellular circumstances that lead to translocation from cytosol to membranes and vice versa. It has been documented that insulin causes mouse lipin phosphorylation in an mTOR-dependent manner in adipocytes. However, nothing is known about it in human immune cells, the residues that become phosphorylated and the kinases implicated. We will explore conditions under which the enzyme become phosphorylated in human macrophages. We will also try to define the specific phosphorylated amino acids and the kinases involved. Besides, by using specific enzymatic assays we will define how phosphorylation affects activity. Once we have gotten all of this information, we will proceed to mutate the relevant residues and study how phosphorylation regulates PAP-1/Lipin1 cellular activity and subcellular distribution. By inhibiting the expression of PAP-1/Lipin1 on human monocytes/macrophages we will study a variety of processes. In the first place, apoptosis related events (just to confirm our previous data with chemical inhibitors). Secondly we will investigate lipid metabolism. The latter will be carried out by different strategies: evaluation of lipid cellular content by mass-spectrometry, and evaluation of the expression of important enzymes involved in lipid metabolism. On the

other hand, if the inhibition of PAP-1/Lipin1 by genetic approaches does promotes apoptosis as is expected, is could be hypothesized that its overexpression could allow not only survival but also cell proliferation. We will verify the correctness of such an assumption. Finally, no immunological studies have been described in animal models where PAP-1/Lipin1 expression levels are extremely different from wild type animals. It is known that inflammation and the insulin resistance that accompanies conditions like metabolic syndrom are closely related. Thus, this aim would mainly focus on the innate immunity of the aforementioned animals. First, we will evaluate the amount and activation levels of peritoneal macrophages in both models, and second, the response of those macrophages against an immune challenge. This aim will help us to address not only the role of PAP-1/Lipin1 in the innate immune system, but also to link obesity/metabolic syndrome and immunity.

3) Subcellular localization studies of lipid-metabolizing enzymes - Ongoing studies are focusing on the localization and stimulus-driven translocation of different members of the phospholipase A_2 (PLA_2) family. These enzymes cleave the fatty acid at the sn-2 position of phospholipids and thus constitute the earliest regulatory point of the eicosanoid biosynthetic cascade. Current studies are being carried out by transfecting chimeric constructs of green fluorescent protein (GFP) or red fluorescent protein (RFP) with the appropriate PLA_2s. GFP and RFP are placed at either the N- or C-termini. of the enzymes. These constructs provide a very useful tool to visualize the intracellular movements of the PLA_2s in response to the different stimuli. Mutagenesis studies are also being conducted to pinpoint the specific amino acids of the PLA_2s that are implicated in the movement among intracellular compartments. For these studies we utilize confocal microscopy.

Transfer of Knowledge

Publication of the results in top professional journals. Our journal of reference; i.e. the journal that sets the standard of excellence in our field, is JBC. We have published much in the past in that journal and expect to keep publishing much there in the future.

Presentation of results at national and international meetings. In keeping with our past, we expect to be regularly invited to present data at top lipid research symposia and conferences.

Objetivos de formación

Difficult to say in advance; that depends too much on external factors, i.e. how many committed students join our lab. Because of the environment we are in, this is not possible to predict with reasonable accuracy. Since we have not been doing too bad in recruiting personnel in the recent past, we speculate that 4-5 PhD theses should be an attainable goal.

Dissemination

We organize an International Workshop on Lipids every two years. Aside from the scientific value, this constitutes an obvious opportunity for divulgation at the level of press, radio and/or TV. In addition, our group maintains a lipid-dedicated website that receives roughly 1000 visits a month. Such a a large audience provides interesting possibilities of dissemination that will undoubtedly be exploited.

Internationalization

"Internationalization" of our scientific work as described in the booklet of instructions is not an immediate priority for us. However, since our group holds significant international

recognition, collaboration proposals from foreign groups are not received infrequently. Our position has traditionally been to accept only those that may bring a definite enrichment to our own research line. At present, we maintain scientific contact with Drs. Dennis (USA), Shirai (Japan), Bianco (Argentina) and Thurnher (Austria). A student of Dr. Bianco spent three months in our lab last spring.

Common Services

We are in the initial stages of establishing a Mass Spectrometry Metabolomics/Lipidomics Service, operated entirely by us. This initiative is supported by the CIBER network we belong to.

Gender Issues

Gender issues pretty much nonexistant. 7 Males and 10 females currently in our group suggests that we are doing fine in this area.

5.3. General Strategy

We lack the ability to predict the future. That said, our general strategy is quite simple: to keep doing exactly as we have been doing in the recent past. Without better personnel and surroundings a leap forward is not possible. As indicated elsewhere, there is very little we could do to improve in these areas given the external circumstances imposed upon us. Following from that, we do not expect critical changes at any level and, since our current standing is good enough, there is no reason not to keep the same path. We will keep doing the experimental approaches which have given us international recognition (the "jewels of the crown" being confocal microscopy and mass spectrometry approaches applied to the study of the molecular cell biology of lipid mediators). These two “jewels of the crown” constitute the state-of-the-art in lipid research. We will keep improving the techniques and bettering ourselves in their use, so as to reach and/or maintain a level of excellence. A new front we are very excited to get into is animal experimentation. We will open research in this area in the near future, and expect it to be productive at short-medium term.

5.4. Strategy Analysis

We understand that our strategy may be perceived by some as generally conservative, but we are also aware that expecting significant improvements to our current situation would simply be unrealistic. As indicated elsewhere, because of how the thing Spanish works our margin of action to (i) exploit our own strengths, (ii) alleviate our weaknesses, (iii) take advantages from the environment and (iv) protect our research from exogenous threats, is practically nil. We are not given independence and resources to effectively make an impact on either of these aspects. What we do is what we can do and, in many respects, we honestly believe we are performing well above our possibilities.

5.5. Follow-up Indicators (Quantitative Objectives)

INDICADORES DE SEGUIMIENTO (OBJETIVOS CUANTITATIVOS)					
	Indicador	2010	2011	2012	2013
Financiación (k€)	Proyectos investigación	100000	100000	100000	100000
	Contratos I+D				
Artículo/Capítulos de Libro (número)	ALTO, Percentil 75	3	3	3	3
	MEDIO, Percentil 50-75				
	BAJO, Percentil <50				
Congresos (número)	ALTO, Percentil 75				
	MEDIO, Percentil 50-75				
	BAJO, Percentil <50				
Libros completos (número)	ALTO, Percentil 75				
	MEDIO, Percentil 50-75				
	BAJO, Percentil <50				
Transferencia (número)	Patentes de prioridad solicitadas				
	Patentes de prioridad licenciadas				
	Spin-offs				
	Indicador	2010	2011	2012	2013
Formación (número)	Tesis defendidas	1	1	1	1
	Cursos (horas)				
Divulgación (número)	Eventos				
	Material				
Internacionalización (número)	Personal extranjero				
	Colaboraciones				
	Artículos en co-autoría				

6. RECURSOS SOLICITADOS

RECURSOS SOLICITADOS > RECURSOS HUMANOS					
Puesto (número)	2010	2011	2012	2013	Total
Científico Titular	0	0	0	0	0
Titulado superior	2	0	0	0	2
Titulado medio	0	0	0	0	0
Ayudante laboratorio	0	0	0	0	0
JAE-Senior	1	0	0	0	1
JAE-Doc	0	1	0	1	2
JAE-Pre	1	1	1	1	4
JAE-Tec	0	0	0	0	0

RECURSOS SOLICITADOS > RECURSOS ECONÓMICOS					
Acción	2010	2011	2012	2013	Total
EQUIPA (€)	400000	0	0	0	400000

Justification

Our subline enjoys a significant international exposure and shows a good record of productivity in terms of both quality and quantity. Ensuring a continuous turnover of personnel is a must to maintaining and eventually improving our performance indicators. That justifies the need for predoc and postdoctoral positions. Intermediate posts (titulado superior and JAE-senior) are sorely needed to "fill the gap" between faculty personnel and students that is produced by the excess of bureaucratic and other non-scientific tasks imposed upon the former. Most of the research we are embarked in requires the use of very sophisticate, and hence, expensive equipment. The resources requested would be destined to acquire and/or replace various pieces of equipment related to our research utilizing mass spectrometry and confocal microscopy techniques.