Innate immunity, Phospholipase A_2s, and Plasmalogens

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June 1, 2023

Our current investigations focus on the enzymes involved in maintaining the composition and levels of different fatty acids in biological membranes, ie phospholipases and acyltransferases. The family of phospholipases A_2 has traditionally constituted the axis of our investigations. These enzymes generate lipids with signaling properties, lysophospholipids on the one hand and free fatty acids on the other, including arachidonic acid. More recently we have focused on the characterization of molecular species of phospholipids that can serve as specific substrates for the action of these phospholipase A_2s in a cellular context. This has led us to ethanolamine plasmalogemes, cell species highly enriched in arachidonic acid. Beyond their involvement in the cellular metabolism of arachidonic acid and other polyunsaturated fatty acids, plasmalogens perform a varied number of functions.

*Instituto Murciano de Investigación Biosanitaria, Murcia, jueves 1 junio 2023 (**Slide 1**).*

Phospholipases A2 are enzymes that act specifically on the ester bond present at the sn-2 position of the glycerol backbone of the phospholipid (Slide 2 – Phospholipase A2 Action on Phospholipids). The reaction gives rise to a lysophospholipid and a free fatty acid, both of which are biologically active. Well, this reaction is of great biological importance, because it has consequences for a large number of cellular processes and functions (Slide 3 – Importance of Phospholipid Hydrolysis by Phospholipase A2). For example, it is a reaction that serves to generate molecular diversity; phospholipase A2 removes one acyl residue, allowing another to enter its place via specific acyltransferases; this is what is known as the Lands cycle and allows cells to be enriched with certain classes of fatty acids, which influences their biophysical properties such as fluidity, etc. Also, phospholipase A2 generates lysophospholipids whose accumulation influences the curvature of a membrane, which is very important for the biogenesis of organelles such as lipid droplets, as we will see in a moment. Phospholipases A2 also help in repair processes, because they eliminate remains of oxidized or truncated fatty acids. And finally, the function that has possibly made these enzymes most famous and the one on which we have focused the most in our laboratory: phospholipases A2¬ are mainly responsible for mobilizing polyunsaturated fatty acids for the synthesis of eicosanoids and fatty acid derivatives. omega-3 such as resolvins, protectins, etc., with key roles in inflammation. A large number of receptors signal by activating intracellular phospholipases A2 that generate lipid messengers (Slide 4 – Role of Phospholipase A2 in Arachidonic Acid Release).

There is a wide variety of phospholipases A2 in eukaryotic cells. Such a variety made it necessary to establish a classification of these enzymes (Slide 4 – Phospholipase A2 Classification). Actually there are several; one of the most useful considers the biochemical properties of these enzymes and is the one shown on this slide (Slide 5 – Phospholipase A2 Classification). According to this classification, phospholipases A2 are grouped into 6 large families, namely (I start here following clockwise): sPLA2 or calcium-dependent secreted enzymes, all of them of low molecular weight; all of them use a common mechanism of catalysis using the His/Asp dyad; cPLA2 or high molecular weight calcium-dependent cytosolic enzymes that use the Ser/Asp catalytic diad; iPLA2, high molecular weight, calcium-independent cytosolic enzymes that use the Ser/Asp catalytic diad; PAF-AH enzymes, whose common characteristic is that they specifically hydrolyze PAF and use the classic Ser/His/Asp catalytic

triad; the L-PLA2 or specific enzymes of lysosomes and the adPLA2 or specific enzymes of adipose tissue. Of all these families today I am going to refer to only two of them: sPLA2 (V) and cPLA2¬ (IV).

We start with the group IVA cytosolic phospholipase A2, commonly known as cPLA2α (Slide 6 – Group IVA Phospholipase A2). It is a very interesting enzyme because it has a little bit of everything and that makes it very attractive from many points of view. The enzyme has two domains, on the left there is a C2 domain in orange, which is a domain for binding calcium and lipids, similar to that found in many proteins, PKCs are a clear example, and on the right the catalytic domain with a structure in form of α/β hydrolase, characteristic of many hydrolases, 8 β sheets connected by 6 α helices. This enzyme, however, has a structural domain that makes it unique: the presence of a double cap that protects the active site. No other hydrolase is known to have a similar capping mechanism. Under resting conditions, this enzyme is soluble, it is found in the cytosol. When cell activation occurs, cytosolic free Ca2+ increases, which causes the enzyme to translocate to membranes thanks to the C2 domain. These two purple dots represent the two Ca2+ atoms required for the translocation. Said translocation can be strengthened and can even occur in conditions of absence of calcium elevations due to the presence in the proteins of two anionic lipid-binding clusters, one to C1P and the other to PIP2. Well, once the enzyme has translocated, the catalytic domain comes into contact with the substrate and it does so by opening the first lid, which are these two α-helices. Once this has occurred, the second cap, in purple, is also removed, exposing the pocket with the catalytic amino acids. A large majority of hydrolases use the Ser/Asp/His triad for catalysis; cPLA2α uses Ser/Asp assisted by Arg. And finally, the third major unique feature of this enzyme is that it has a tremendous predilection for phospholipids with AA, which does not occur with any other lipase. This great predilection is a consequence of its very particular active site that only accommodates well polyunsaturated residues in the cis configuration. In particular the presence of a double bond at C5 seems to play an important role in substrate recognition. It is important to emphasize that this does not mean that the enzyme cannot hydrolyze phospholipids without AA; it will, but a much slower pace. Finally, another important feature of this enzyme is that it is phosphorylated at Ser505, which is indicated by this light blue dot in the figure. This phosphorylation is necessary for the enzyme to display full activity, and is carried out by members of the MAPK, ERK, p38, and JNK family, and appears to be very important not so much for increasing enzyme activity as for maintaining an optimally active configuration that allow it to translocate to the membrane in question. Depending on the cell type and the stimulus the MAPK involved varies. Studies with GFP chimeras have shown that cPLA2α translocates very markedly to perinuclear membranes (Slide 7 – Translocation of cPLA2α to Perinuclear Membranes), but more exhaustive and careful studies have shown that the enzyme is quite promiscuous and can also translocate to other intracellular membranes. For example, in phagocytic cells, the enzyme translocates to the phagosome, as we will see later.

This figure shows how important cPLA2α is to the receptor-mediated process of archidonic acid mobilization. These are RAW264.7 macrophage-like cells and stimulated to study fatty acid release, in this case with a TLR3 activator (although macrophages respond to many different stimuli by releasing arachidonic acid). To study said release, the cells were labeled with radioactive fatty acid and, at different times seen with the radioactive label, it was released from the cell into the extracellular medium. This is how arachidonic acid release was previously measured; these are old experiments, before we had a mass, which is how we do it now (Slide 8 – cPLA2α-Dependent Arachidonic Acid Mobilization). It can be seen to the right that activation of the release is accompanied by phosphorylation of the enzyme. And when we inhibit cPLA2α by chemical means or by siRNA knock-down, the response is completely inhibited. These data demonstrate the fundamental role of cPLA2α in the process. These experiments are relatively old and were done by labeling cells with radioactive fatty acid. It was precisely in those years that the great lipidomic revolution arose and the increasingly widespread use of mass spectrometry for lipid analysis. We were lucky to ride that wave and implement this technology in our laboratory, which gave us the great opportunity to analyze defined molecular species. This would allow us to define the substrates at the molecular level in a cellular context; that is, to try to identify possible species of phospholipids that are hydrolyzed by PLA¬2α and thus characterize the substrate specificity of the enzyme in the cell (Slide 9 – Mass Spectrometry:

Defined Molecular Species).

And at this point, it is important to remember that not all membrane glycerophospholipids are the same, something that will sound very familiar to this audience (Slide 10 – Not All Glycerophospholipids…). 80% of the total membrane glycerophospholipids contain two fatty acids, but 20-25% do not, they only contain one, because in the sn-2 position they contain a fatty alcohol (Slide 11 – Not All Glycerophospholipids…). And among these there are those with a double bond conjugated to the oxygen of the ether bond; They are plasmalogens. In innate immune cells, ethanolamine plasmalogens are very abundant: they account for 70-75% of the total ethanolamine glycerophospholipids. And what is more important for our interests, they constitute an important cellular reservoir of polyunsaturated fatty acids in the cells of the immune system. This figure shows the fatty acid composition of mouse peritoneal macrophage ethanolamine plasmalogens (Slide 12 – Plasmalogen Fatty Acid Composition in Murine Macrophages). Depending on the nature of the fatty alcohol in sn-1, there are three types of plasmalogens: those containing palmitic alcohol, oleyl alcohol, and stearic alcohol. It can be clearly seen that, in all cases, almost all that is present is polyunsaturated, with arachidonic acid being by far the most common fatty acid. And if, going a little further, we look at the global distribution of arachidonic acid species among all types of macrophage phospholipids, it can be seen that plasmalogens are among the richest species in this acid (Slide 13 – Arachidonic Acid- Containing Species in Murine Macrophages). Therefore, it would seem logical to suggest that plasmalogens must constitute an important source of free arachidonic acid during cell activation (Slide 14 – Plasmalogens Must Constitute…).

For these studies we were fortunate to have plasmalogen-deficient cells, generously donated by Dr. Raphael Zoeller of Boston University. Cells were prepared by chemical mutation and clonal selection. This is the biosynthetic pathway of plasmalogens in mammalian cells, with the first part in the peroxisome and the rest in the endoplasmic reticulum (Slide 15 – Plasmalogen Biosynthesis in Animal Cells). We have two mutant cells from the RAW macrophage cell line. The first, called RAW.108, lacks the first enzyme in the pathway (Slide 15 – Plasmalogen Biosynthesis in Animal Cells), and the second, called RAW.12, lacks the same enzyme. and also the final PEDS-1 desaturase that will be so familiar to many of those present (Slide 16 – Plasmalogen Biosynthesis in Animal Cells). The first thing we did was confirm by LC-MS that indeed these cells lacked plasmalogens. It can be seen from this slide (Slide 17 – Phospholipid Species Composition of Plasmalogen-Deficient Cells) that the two mutant cells lacked not only plasmalogens but also PC ethers, as expected. What is interesting here is that there is a compensatory elevation in the levels of AA-bearing diacylphospholipids, so the end result is that the mutants contain the same levels of AA and other fatty acids as normal cells. The total amount of cellular arachidonic acid and its distribution by phospholipid class, as measured by GC-MS, does not change either (Slide 18 – Phospholipid Fatty Acid Composition of Plasmalogen-Deficient Cells). Therefore, it seems clear that the mutants compensate for their deficiency in ether phospholipids by producing more diacylphospholipids bearing arachidonic acid, which, when considered, makes quite a bit of sense. In the following experiments we measured the release of AA in these cells after stimulation with a classical stimulus such as zymosan and, to our surprise, the mutants released the same amount of fatty acids as normal cells (Slide 19 – AA Release by Plasmalogen - Deficient Cells). And in line with these data, there was also no difference in eicosanoid production, both in quantity and quality (Slide 20 – Eicosanoid Production by Plasmalogen-Deficient Cells). Thus, the obvious conclusion of these experiments, as surprising as it is unexpected, is that plasmalogens have no influence on the arachidonic acid mobilization response or its subsequent conversion to eicosanoids (Slide 21 – Cellular Plasmalogen Status Has No Influence…). Which leaves us, among other things, with the unanswered question of what the plasmalogens can be used for, what biological role they have.

And the search for an answer to this question leads us to the second story about plasmalogens that I want to tell today, which deals with phagocytosis (Slide 22 – Macrophage Polarization and Phagocytosis). Depending on the environment, macrophages can be in two different states of activation, which is called polarization (Slide 23 – Polarized Activation of Macrophages). On the one hand, there is the classic or M1 response, which leads macrophages to acquire a proinflammatory phenotype and is triggered by stimuli such as LPS or IFNγ and leads

to the secretion of proinflammatory cytokines such as those indicated in the figure; and on the other hand there is the M2 or alternative, anti-inflammatory, pro-resolving response, IL-4 is the typical stimulus and finally leads to the positive regulation of anti-inflammatory genes such as IL-10, TGFα or ARG-1 among others. Well, the first thing that occurred to us was to think about what would happen to the expression of phospholipase A2 in macrophages under polarization conditions. Then, we stimulated the cells with LPS + IFNγ to obtain M1 macrophages and IL-4 to obtain M2 macrophages (Slide 24 – Expression of PLA2s During Human Macrophage Polarization) and then we analyzed by qPCR the expression of the different genes. And what we found was something unexpected. Only one phospholipase A2 varied significantly, sPLA2-V, and it did so under M2 conditions. It was verified by immunoblot that the induction of the sPLA2-V gene leads to a time-dependent increase in the protein (Slide 25 – Induction of sPLA2-V Protein During Macrophage Activation by IL-4). To verify if this induction is something particular to IL-4 or is a general feature of M2 polarization, we next tested other stimuli that also polarize M2, such as C-MSF and IL-10 (Slide 26 – Induction of sPLA2-V Protein by Other M2 Stimuli). In both cases, sPLA2-V increased quite clearly, indicating that sPLA2-V is indeed a good marker of M2 activation status. Well, on the next slide I present to you sPLA2-V, the second phospholipase A2 that I am going to talk about today (Slide 27 – Group V Phospholipase A2). It is very different from the one I presented before.

What are the functional consequences of this IL-4-induced increase in sPLA2-V? One of the most evident functional characteristics of M2-polarized macrophages is their increased phagocytic capacity. Therefore, we analyzed the ability of these cells to phagocytose zymosan, which is a homogenate of the cell wall of the yeast S. cerevisiae in the presence and absence of sPLA¬2-V (Slide 28 – sPLA2-V Depletion Inhibits IL-4- Stimulated Zymosan Phagocytosis). As can be seen on the slide, IL-4 treated cells phagocytose much more zymosan than untreated cells. When cells deficient in sPLA2-V were used, such an increase is not observed. The next slide shows the opposite experiment, the effect of overexpressing sPLA2-V on the phagocytic capacity of cells (Slide 29 – sPLA2-V Overexpression Increases Zymosan Phagocytosis). It can be seen that, simply by overexpressing the enzyme, the phagocytic capacity of the cells increases as much as if the cells had been treated with IL-4, thus making IL-4 treatment unnecessary to observe the same level of response.

sPLA2-V is an enzyme and therefore the obvious question to investigate would be to verify if these increases in the phagocytic capacity of cells are related to changes in the phospholipid content of cells (Slide 30 – Do these effects correlate with changes in phospholipid content?). To answer this question, we proceeded to analyze the phospholipid content of these cells using liquid chromatography coupled with mass spectrometry. We used cells deficient in sPLA2-V by siRNA. The next slide shows the cellular content of the major PC species (Slide 31 – Choline Phospholipid (PC) Species in IL-4-Treated Human Macrophages) in cells treated or not with IL-4 and with normal or reduced levels of sPLA2- v. In no case were we able to appreciate any significant difference. We also did not observe anything appreciable when we analyzed ethanolamine (Slide 32 – Ethanolamine Phospholipid (PE) Species in IL-4-Treated Human Macrophages) or inositol (Slide 33 – Phosphatidylinositol (PI) Species in IL-4-Treated Human Macrophages) phospholipids. These experiments were done in human macrophages, which are very large cells and have an enormous amount of lipids; therefore if the response to IL-4 is not very extensive, the effect would be offset by the large amount of lipid present. Finally, it occurred to us to look at the cellular content of lysophospholipids, which are a direct product of the action of sPLA2-V and, furthermore, their cellular content is necessarily lower (Slide 34 – Lysophospholipid Species in IL-4-Treated Human Macrophages). Again, no differences were observed between IL-4-treated versus control cells; however, when we examined the sPLA2-V deficient cells, a very striking change was observed, highlighted by the red arrows. Levels of all lysoPE species were significantly decreased. Note that the decrease in lysoPE levels is observed in IL-4-treated cells, but not in untreated cells, indicating that this decrease is related to the activation state of the cell. In other words, in cells treated with IL-4, sPLA2-V is necessary to maintain lisoPE levels (Slide 35 – LysoPE levels are maintained by sPLA2-V in IL-4-treated cells).

So what is the biological consequence of this finding? To answer this we went back to our phagocytosis assay and

what we tried to determine was if the addition of exogenous lysoPE (a lysoplasmalogen was used!) has any effect on phagocytosis (Slide 36 – LysoPE Restores Phagocytosis in sPLA2-V-Deficient Cells -Zymosan). As before, IL-4 increased phagocytosis of zymosan particles and the presence of lisoPE did not show any significant effect. But what happens if we use cells deficient in sPLA2-V? (Slide 37 – LysoPE Restores Phagocytosis in sPLA2-V-Deficient Cells - Zymosan). The IL-4 response is inhibited and the addition of lisoPE almost completely restores the response, so lisoPE replaces sPLA2-V under these conditions. This experiment was repeated using this time live bacteria, E. coli, as a phagocytic stimulus, and the results were the same, in fact it looks even better; lisoPE restored the IL-4 effect in sPLA2-V-deficient cells (Slide 38 – LysoPE Restores Phagocytosis in sPLA2-V-Deficient Cells - Bacteria). The next slide shows that this effect of lisoPE is specific, since it is not reproduced by any other lysophospholipid, lysoPC or lisoPI (Slide 39 – LPC and LPI Do Not Restore Phagocytosis in sPLA2-V-Deficient Cells). Therefore, as a conclusion to this part of the talk, we have seen that lysoPE plays a key role in increasing the phagocytic activity of M2-polarized macrophages and that this metabolite is produced by sPLA2-V (Slide 40 – LysoPE is involved in IL-4-induced phagocytosis).

To deepen these effects of sPLA2-V, we think it might be important to investigate the compartmentalization of lysoPE synthesis, that is, in which part of the cell this lysoPE is produced or, in other words, to determine the subcellular localization of the enzyme. that is producing it. For this purpose we used cells transfected with sPLA2-V bound to EGFP (Slide 41 – sPLA2-V Does Not Translocate to the Phagosome in Human Macrophages). In the resting cell it can be seen that the cytoplasm is dotted with green dots that probably represent secretory granules. When we treat the cells with zymosan to start phagocytosis, the green dots of sPLA2-V in the cytoplasm disappear most likely because the enzyme is being secreted, but we do not see any accumulation of green around the particles, suggesting that the enzyme is being secreted. enzyme does not significantly interact with the phagosome. This is something that surprised us, since previous work by others had shown that sPLA2-V translocates to the phagosome in mouse macrophages. Another notable difference between human and mouse cells.

This behavior of sPLA2-V is also in stark contrast to that of cPLA2α, which we return to the discussion, which translocates to the phagosome (Slide 42 – cPLA2α Translocates to the Phagosome in Human Macrophages). These are EGFP-cPLA2α transfected human macrophages, exposed to zymosan in red. The movement of the enzyme into the phagosome can be clearly seen, which is even better appreciated in pseudocolor. And since we are dealing with cPLA2α, we will say that if what we transfect is a mutant cPLA2α that cannot be phosphorylated because we have replaced Ser505 with an Ala, the enzyme does not translocate (Slide 43 – S505A cPLA2α Mutant Does Not Translocate to the Phagosome), which demonstrates the importance of translocation for this enzyme to manifest its functionality. In the upper part of the figure, the translocation of the enzyme is shown, as in the previous one. But if an S505A mutant is used, which prevents phosphorylation, the enzyme does not translocate at all.

And well, what is cPLA2α doing in the phagosome? The results we have in this regard are certainly surprising (Slide 44 – cPLA¬2α Inhibition Modifies the Pattern of Phagosome Internalization). A macrophage is shown at the top after 2 h of exposure to zymosan; the phagocytosed particles appear to be concentrated inside the cell, around the nucleus. However, if we use pyrrophenone, a cPLA2α inhibitor, there are many particles around the nucleus, but there are also many scattered around the cytoplasm. And if we use cells deficient in cPLA2α by siRNA, we see the same thing, many cells scattered around the cytoplasm. Here on the right is the quantization. With all this we can show a scheme like the one shown in this slide (Slide 45 – Distinct Roles for sPLA2-V and cPLA2α in Regulating Phagocytosis), which indicates that there are two phospholipases A2 involved in phagocytosis in human macrophages. On the one hand, the sPLA2-V that we do not know exactly where it is working. We speculate perhaps in the plasma membrane, near the phagosome, where it hydrolyzes PE to generate lysoPE, which is necessary to regulate the spread or quantity of ingested particles. On the other hand, we have cPLA2α, which interacts with the phagosome and somehow regulates the internalization of the particles. And one last question that I would like to address in this topic is if these two enzymes interact, if there is some kind of cross-talk between them. And the answer is yes, there is interaction. If we take cells deficient in sPLA2-V by

siRNA, put them to phagocytose zymosan and examine the phosphorylation status of cPLA2α, we find that there is a significant decrease; here on the right is the quantization. So this is very interesting because if sPLA2-V regulates cPLA2α phosphorylation and cPLA2α phosphorylation is important for translocation to the phagosome, sPLA2-V is regulating cPLA2α translocation to the phagosome (Slide 46 – sPLA2-V Depletion by siRNA Inhibits cPLA2α Phosphorylation). Therefore as a conclusion to this part, we see that there are two phospholipases regulating phagocytosis but their function is not redundant; one regulates the extension of the process and the other internalization (Slide 47 – Two Distinct Phosholipase A2s Regulate Phagocytosis in a Non-Redundant Manner).

And now for the last part of the talk, we return to our plasmalogen-deficient cell lines in order to dig deeper into the role of this class of phospholipids. RAW cells are not very good at phagocytosing zymosan particles, so, as was done with previous experiments with human macrophages, it is necessary to opsonize the stimulus to get good responses. The mutants phagocytosed zymosan to a much lesser extent than normal cells, which is not particularly surprising (Slide 48 – Phagocytosis of Zymosan by Plasmalogen-Deficient Cells). And what happens when the phagocytosis assay is performed with cells that have been treated with lisoPE (lysoplasmalogen vs lysophosphatidylethanolamine), in this case? (Slide 49 – LysoPlsEtn Increases Phagocytosis in Plasmalogen-Deficient Cells). Yes, the answer is fully restored. These experiments are also notable because, under the same conditions, (acyl) lysoPE has no effect, so it is clear that the vinyl ether bond here is crucial.

Next, in order to continue exploring the effects of plasmalogens on our macrophages, we performed FRAP (fluorescence recovery after phostobleaching) experiments using confocal microscopy to analyze membrane fluidity (Slide 50 – Analysis of Membrane Fluidity in Macrophages - FRAP). Basically what you do here is burn a defined area of the cell membrane and then measure the recovery of fluorescence over time. The more fluid a membrane is, the less time it will take to recover fluorescence. It can be seen here that the mutant cells recover more fluorescence than the normal ones, thus indicating that their membranes are more fluid. From these measurements a "mobile fraction (fluorescence recovery)" can be calculated such that the higher this value, the more fluid the membrane. When RAW.108 cells were exposed to lysoPlsEtn but not lysoPtdEtn, the mobile fraction of the membrane decreased, reaching values similar to those of normal RAW 264.7 cells. These data demonstrate that increased plasmalogen levels in RAW.108 cells reduce cell membrane fluidity to levels found in cells showing normal plasmalogen levels (Slide 51 – Analysis of Membrane Fluidity in Macrophages - FRAP). That is, the plasmalogens contribute to rigidify the membranes.

And consistent with these data, it has been suggested that ethanolamine plasmalogens are frequent constituents of membrane lipid rafts, which are membrane microdomains that compartmentalize cellular processes and serve as organizing centers for the assembly of signaling molecules (Slide 52). – Plasmalogens Accumulate in Lipid Rafts). It has also been reported that lipid rafts can be visualized with Alexa Fluor 647 labeled cholera toxin B subunit (CT-B), which binds to the GM1 ganglioside present in lipid rafts. Thus, in the following series of experiments, a confocal microscopy analysis of macrophage lipid rafts was carried out to determine the influence of plasmalogen content on these structures, as well as its relation to phagocytosis. This figure shows that treatment of RAW 264.7 cells or RAW.108 cells with lisoPlsEtn, but not lisoPtdEtn, increased both the number and size of lipid rafts in the two cell types in a similar manner (Slide 53 – LysoPlsEtn Increases the Number and Size of Lipid Rafts - Unstim). The same result was obtained with phagocytizing cells (Slide 54 – LysoPlsEtn Increases the Number and Size of Lipid Rafts – Latex Beads). In these phagocytosis experiments, opsonized latex beads were used as a stimulus instead of zymosan, because the latter's autofluorescence interferes with the Alexa Fluor 647-labeled CT-B signal.

And finally, again in line with the above, treatment of RAW.108 cells with lisoPlsEtn increased the phosphorylation/activation of p44ERK and p42ERK kinases, suggesting that plasmalogens enhance intracellular signaling originating from phagocytic receptors, which may be decisive for optimal phagocytosis (Slide 55 – Lysophospholipid Effects on MAPK Signaling).

So, as a conclusion to my talk, we can say that, contrary to all expectations, the presence or absence of plasmalogens in cells does not exert any influence on the eicosanoid production response of macrophages. But on the other hand, plasmalogens seem to be key to phagocytosis. Plasmalogen levels determine properties of membrane lipids that may be essential for an adequate phagocytic response (Slide 56 – Cellular Plasmalogen Status Determining Specific Responses). There is therefore a biological specificity on which we must continue investigating.

To conclude, thank the "plasmalogen crew" of my laboratory... (Slide 57 - Acknowledgments). Thank you also to our collaborators and sponsors (Slide 58 - Acknowledgments). A comprehensive list of significant papers from our laboratory, directly related to the topic under discussion, follows.

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