Hospitalaria

Revisión

Role of oleic acid in immune system; mechanism of action; a review

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Abstract

Introduction: **Although n-3 polyunsaturated fatty acids have been widely described as anti-inflammatory fats, little is known about the role of oleic acid in immune system.**

Aim: **The aim of the present review is to join all the reports available in order to analyze where exactly the knowledge concerning this topic is and what the causes of the controversial data could be.**

Methods: **We searched electronic databases and bibliographies of selected articles were inspected for further reference.**

Results: **Diets rich in oleic acid have beneficial effects in inflammatory-related diseases. In addition, a wide range of studies evaluate the effect of oleic acid in different cellular functions thus reporting a potential mechanism for the biological effect of such a fat. However, some controversial data can be found in literature, maybe related to the kind of study or even the dose of the reagent added.**

Conclusion: **In conclusion, oleic acid could be reported as an anti-inflammatory fatty acid playing a role in the activation of different pathways of immune competent cells.**

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Key words: *Oleic acid. Immune system. Intracellular signaling.*

PAPEL DEL ACIDO OLEICO EN EL SISTEMA INMUNE; MECANISMO DE ACCIÓN; REVISIÓN CIENTÍFICA

Resumen

Introducción: **Los ácidos grasos poliinsaturados de la familia n-3 han sido ampliamente caracterizados por su potencial antiinflamatorio. Sin embargo, las evidencias relativas al papel del ácido oleico en el sistema inmune son escasas.**

Objetivo: **El objetivo de la presente revisión bibliográfica es hacer una recopilación de todos y cada uno de los trabajos publicados a este respecto, al objeto de evaluar dónde se encuentra el conocimiento relativo a esta área y cuáles pueden ser las causas de los resultados contradictorios.**

Métodos: **Se ha realizado una búsqueda bibliográfica a través de bases de datos electrónicas y las referencias de los artículos de interés han sido utilizadas como fuente de búsquedas más avanzadas.**

Resultados: **Las dietas ricas en ácido oleico parecen estar asociadas con un beneficio en determinadas patologías de base inflamatoria. Además, un gran número de estudios se han centrado en evaluar el papel que juega tal ácido graso en distintas funciones celulares, argumentando posibles mecanismos que sustentarían los efectos biológicos que se atribuyen a su consumo. Sin embargo, en algunos casos se observan resultados contradictorios que quizá puedan deberse al tipo de estudio desarrollado o incluso a la dosis de ácido con la que se experimenta.**

Conclusión: **En conclusión, el ácido oleico podría ser presentado como una grasa anti-inflamatoria dado el papel que juega en la activación de distintos mecanismos de señalización de células inmunocompetentes.**

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Palabras clave: *Acido oleico. Sistema inmune. Señalización celular.*

Abbreviations

PUFA: Polyunsaturated fatty acid.
OA: Oleic acid.
MUFAs: Monounsaturated fatty acids.
VCAM-1: Vascular cell adhesion molecule-1.
ICAM-1: Intercellular adhesion molecule-1.
NF-κb: Nuclear factor-kappa B.
AA: Arachidonic acid.
EPA: Eicosapentanoic acid.
ROS: Reactive oxygen species.
$O_2^{\cdot-}$: Superoxide.
PL: Phospholipase.
IP_3: Inositol,1,4,5 triphosphate.
DAG: Diacylglycerol.
$[Ca^{2+}]i$: Intracellular Ca^{2+} concentration.
PKC: Protein kinase C.

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HOSO: Sunflower oil.
NK: Natural killer.
TCR: T cell receptor.
AICD: Activation-induced cell death.
PA: Phosphatidic acid.
DHA: Docosahexaenoic acid.
PIP_2: Phosphatidilinositol 4,5 biphosphate.
PI-PLC: Phosphatidylinositol-specific PLC.
polyDAGs: Polyunsaturated DAGs.
monoDAGs: Mono-unsaturated DAGs.
satDAGs: Saturated DAGs.
PI: Phosphatidylinositol.
PS: Phosphatidylserine.
PC: Phosphatidylcholine.
PE: Phosphatidylethanolamine.
SOG: 1-stearoyl-2-oleyl-sn-glycerol.
Stromal Interaction Molecule 1: STIM1.

Introduction

The first evidence of the beneficial effect of fatty acids in the human immune system and in inflammatory processes comes from epidemiological studies on Greenland Eskimos, who presented a low rate of several inflammatory-related diseases, which was directly linked to their high levels of n-3 polyunsaturated fatty acid (PUFA) intake. By contrast, the normal diet in various parts of the world contains other unsaturated fatty acids; an example is the widespread use of olive oil, rich in oleic acid (OA), in Mediterranean countries. OA is not only abundant in certain diets but is also the most important constituent of plasma free fatty acids. However, all the scientific effort has been mainly focused on the study of n-3 PUFA, letting the research concerning the effect of the monounsaturated OA be overshadowed. In general, the n-6 PUFA are believed to enhance immune function whereas the n-3 PUFA suppress it. Olive oil has classically been used as a placebo treatment in studies investigating the effects of fish oils on immune function, because monounsaturated fatty acids (MUFAs) were typically regarded as being neutral fatty acids. Nevertheless, there is evidence that MUFA-rich oils have effects which are similar to the effects of fish oils on animals. The studies focusing on the immunomodulatory properties of MUFA have, therefore, reported controversial results, thus the aim of the present review is to join them all in order to analyze where exactly the knowledge concerning this topic is and what the causes of the controversial data could be.

Biological effects of oleic acid in the immune system

The Mediterranean Diet appears to be effective in reducing coronary atherosclerosis and the risk of fatal complications like sudden cardiac death.[1] Both fish oil and olive oil have been reported to lower plasma fibrinogen in women with high baseline fibrinogen concentrations in a double-blind crossover study.[2] Other studies have demonstrated no significant difference between fish-oil supplements and olive oil in preventing restenosis after coronary angioplasty.[3] It has been also suggested that the consumption of olive oil may have beneficial effects on rheumatoid arthritis and it has been proposed that the suppressive effect of olive oil on the development of these pathologies may be exerted via an effect on the immune system.[4-6]

As described above, diets rich in MUFA have been linked with a low prevalence of inflammatory disease. As cells of the immune system are an inherent part of the inflammatory events involved in the development and progression of inflammatory disease, we will describe the influence of OA in different aspects of immune cells.

Materials and methods

Search strategy

We consulted studies published in electronic databases such as Pubmed or Medline. The bibliographies of selected articles were inspected for any further reference.

Firstly, we studied the title and abstract of all kind of papers (regular or review papers) with a potential interest to understand the role of oleic acid in immune system. We mostly focused on those related to the mechanisms of action of such a fatty acid at the cellular level. Thus, the main key words used in the search were: "oleic acid", "immune system", "neutrophils", "lymphocytes", "intracellular signaling". Then, the text of the main trials that met the criteria previously mentioned was fully examined to extract the specific data included in the review.

Results and discussion

The innate immune system: key role of neutrophils

Effect of oleic acid on neutrophil effector functions

Adhesion, migration and phagocitosis

Neutrophils play a pivotal role in the defense of the human body against infections. However, overwhelming activation of neutrophils is known to elicit tissue damage. Numerous studies in literature evaluate the effects of OA on leukocyte adhesion. In vitro studies have shown that treatment with micromolar amounts of oleate inhibited the endothelial expression of the vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and the intercellular adhesion molecule-1 (ICAM-1) in several endothelial cells.[7-11] Other authors found that OA had no effects on human endothelial

cells or on the leukocyte adhesion molecule.[12] In any case, a lack of proinflamatory effects of OA has been reported. The previous findings are supported by studies *in vivo* demonstrating that a meal rich in OA beneficially modulates postprandial soluble ICAM-1 and VCAM-1.[13] These data constitute an additional explanation for the beneficial effects of OA, exerted through the inhibition of the very early phenomena in atherogenesis, by modulating endothelial activation through the expression of the gene products involved in leukocyte recruitment, thus confirming the link between OA and cardiovascular disease complications.

In addition, OA has the ability to reduce the inflammatory effects of long-chain saturated fatty acids in human aortic endothelial cells. In this sense, this fatty acid inhibited the stearic acid-induced increase in ICAM-1 expression as well as stearic acid-induced phosphorylation of nuclear factor-kappa B (NF-κB), a transcriptional regulator of ICAM-1.[14]

Studies in other cell lines have also corroborated the findings above stated. Thus, ICAM-1 expression of non-stimulated and cytokine stimulated Caco-2 cells cultured for 22 days with arachidonic acid (AA), was significantly higher as compared to eicosapentanoic acid (EPA) and OA, suggesting that the replacement of AA by EPA or OA in the colon mucosa might have beneficial effects for inflammatory bowel disease patients.[15]

By contrast, OA has been also reported to increase the cell surface expression of CD11b and induce the high affinity state of this integrin. This MUFA, through a CD11b-mediated mechanism, induces neutrophil aggregation and neutrophil-endothelial cell attachment.[16]

Only a few studies are available concerning the effect of OA on leukocyte migration. Ferrante et al. demonstrated that OA was able to inhibit leukocyte-migration but its effect was far from the one exhibited by PUFA.[17]

Conflicting results can be found concerning other leukocyte functions. Whereas some researchers have reported that OA enhanced neutrophil phagocytic capacity and candidacidal activity,[18,19] others show that this fatty acid caused no changes in bactericidal activity and only moderated decreases in phagocytosis and chemotaxis in very high concentrations.[20]

Reactive oxygen species (ROS) production

Once they have arrived at the damaged tissue and engulfed the pathogen, activated neutrophils secrete several cytotoxins, such as superoxide ($O_2^{\bullet-}$), the precursor of other ROS, granule proteases and bioactive lipids.[21,22] ROS production is related to the killing of invading microorganisms, but it can also directly or indirectly cause damage by destroying surrounding tissues. Thus, it is of utmost importance to clarify the effect of fatty acids on neutrophils respiratory burst.

Numerous studies have reported the ability of unsaturated fatty acids to influence ROS production in neutrophils.[18,23-32] However, depending on the experimental conditions, fatty acids can inhibit, enhance or even synergize neutrophil activation. For example, some studies reported that C18 fatty acids inhibit ROS generation.[31] By contrast, others demonstrated an important interaction between fatty acids and cytokines, showing a markedly augmented amount of superoxide produced in response to fatty acids in TNF-pretreated neutrophils.[28] In any case, most of the studies in literature have reported an increased ROS generation by unsaturated fatty acids-stimulated neutrophils. Within these, all reports establish differences between fatty acids in their ability to induce ROS generation in unstimulated neutrophils, suggesting that fatty acids distinctively influence neutrophil function depending on the fatty acid structure. Nevertheless, contradictory observations can be found in this respect, which may be in part the result of the different methods used in the determinations.[26]

Mechanisms undergoing OA effects

As described in the previous section, numerous studies have shown that unsaturated fatty acids stimulate $O_2^{\bullet-}$ release from neutrophils and macrophages. However, the mechanism by which these agents exert its effect is complex and has been the subject of several studies.

Perturbation of neutrophils membrane, either by phagocytosis or by different agents stimulates a number of responses which includes activation of an NADPH oxidase. Stimulation of this oxidase leads to the production of large quantities of ROS. Free fatty acids are known to activate NADPH-oxidase in the cell-free $O_2^{\bullet-}$ generating system.[32,33] Robinson et al. proposed a mechanism for the fatty acid-induced respiratory burst related, at least in part, to its ability to activate phospholipase A_2 (PLA_2).[34] The endogenous AA liberated by PLA_2 action on membrane phospholipids may be in a compartment that effectively activates the NADPH oxidase which is responsible for the respiratory burst. These findings (the direct activation of PLA_2 by fatty acids) are consistent with previous reports demonstrating that fatty acid-induced ROS production, and various other biological responses, in leukocytes, are due to the fatty acids themselves rather than to cyclooxygenase or lipoxygenase metabolites.[17,35-38] By contrast, the latter reports argue against those suggesting that fatty acids can be metabolized by lipoxygenase to hydroperoxides which are intermediates in the release of $O_2^{\bullet-}$.[39,40]

Some researchers have detected the expression of a GPCR40 in bovine neutrophils. The same group, hypothesized that OA could modulate bovine neutrophil responses and these responses could be induced by GPR40 activation.[41] By contrast, others have suggested

Table I
Key studies evaluating the effect of OA in innate immune system

Study	*Key findings*
Neutrophil functions	
Carluccio MA et al.; Massaro M et al.; Sanadgol N et al.; Christon RA; De Caterina R et al.[7-11]	OA inhibits the endothelial expression of VCAM-1, E-selectin and ICAM-1
Hoithe MR et al.[12]	OA has no effect on leukocyte adhesion molecule
Pacheco YM et al.[13]	A meal rich in OA beneficially modulates postprandial ICAM-1 and VCAM-1
Harvey KA et al.[14]	OA inhibits stearic-induced increase in ICAM-1 as well as scaric acid-induced phosphorylation of NF-κb, a transcriptional regulation of ICAM-1
Mastrangelo AM et al.[16]	OA induces neutrophil aggregation and cell attachment
Ferrante A et al.[17]	OA inhibits leukocyte migration but its effect is far from that exhibited by PUFA
Padovese R et al.; Martins de Lima-Salgado T et al.[18,19]	OA enhances neutrophil phagocytic capacity and candidacidal activity
Hawley HP et al.[20]	OA has almost no effect in bactericidal and phagocytosis activity
Padovese R et al; Badwey JA et al.; Morimoto YM et al.; Hatanaka E et al.; Yamaguchi T et al.; Li Y et al.; Hardy SJ et al.; Juttner B et al.; Hwang TL et al.; Tanaka T et al.[18,23-32]	OA modulates neutrophil ROS production
Mechanisms involved	
Hidalgo MA et al.[41]	OA modulates neutrophil responses through GPR40 activation
Hardy SJ et al.[52]	There is a Ca^{2+}-independent mechanism behind the ROS production induced by OA
Morimoto YM et al.; Hidalgo MA et al.[25,41]	There is a Ca^{2+}-dependent ROS production induced by OA
McPhail LC et al.[53]	OA stimulates PKC activation in vitro
Carrillo C et al.[51]	OA induces a PKC-dependent ROS production
Padovese R et al.; Hardy SJ et al.[18,29]	OA induces a PKC-independent ROS production

that unsaturated fatty acids do not act as other stimulating agents which bind to neutrophils at specific receptors. The latter proposed that they may exert their effects by intercalating into and disordering regions of membranes,[42,43] thus affecting proteins involved in $O_2^{\bullet-}$ production that are associated with those regions.[24] According to these authors, a component involved in $O_2^{\bullet-}$ production appears to be sensitive to transitions in the bilayer. A potential component of this pathway could be phospholipase C which may be activated by phase transitions in the membrane induced by cis-unsaturated fatty acids.[24,44] Inositol,1,4,5 triphosphate (IP_3) and diacylglycerol (DAG) are two products of this lipase. The former leads to the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) whereas the latter is the physiological activator of protein kinase C (PKC).[45] Both mobilization of intracellular Ca^{2+} and PKC activation have been postulated as potential mechanisms behind the ROS generation event induced by fatty acids. However, although numerous studies can be found in this respect, contradictory are their results.

Fatty acids, herein OA, have been reported to alter Ca^{2+} homeostasis in different immunocompetent cells.[46-51] However, the relationship between OA-induced ROS production and Ca^{2+} mobilization is complex and the precise mechanism of action is still unclear. Some authors have reported a Ca^{2+}-independent mechanism behind the ROS production.[52] By contrast, other studies have shown a link between these two pathways.[25,41] Other studies available focus on the role of Ca^{2+} ion in the extracellular medium, suggesting an inhibitory effect of Ca^{2+} on the fatty acid-induced $O_2^{\bullet-}$ generation, attributed to the ionic interaction between the carboxyl group of the fatty acid and the Ca^{2+}.[27]

In addition, the involvement of a PKC is also controversial.[18,25,52] A role for PKC in the stimulation of neutrophils has been proposed on the basis of the ability of various activators of this enzyme to elicit different cellular responses in neutrophils.[53-55] As unsaturated fatty acids stimulate PKC in vitro, a role for fatty acids as "second messengers" in the regulation of $O_2^{\bullet-}$ production has been thus proposed.[53] By contrast, several reports failed to demonstrate a link between PKC activation and ROS production.[18,29] Moreover, there are also findings on the PKC-mediated capacity of some fatty acids to enhance the PMA-stimulated $O_2^{\bullet-}$ production at nanomolar concentrations and to depress it at micromolar concentrations.[56]

Table II
Key studies evaluating the effect of OA in adaptative immune system

Study	*Key findings*
T-cell functions	
Virginia R et al.[57]	OA inhibits Jurkat T-cell proliferation as well as IL-2 and INF-gamma production
Llado V et al.[58]	Minerval inhibits T cell proliferation
Yaqoob P et al.; Calder PC et al.; Jeffery NM et al.[59,60-63]	A diet rich in OA inhibits spleen and lymph node induced-proliferation
Yaqooh P et al.[65]	A MUFA-rich diet does not affect the proliferative response of cells
Cury-Boaventura MF et al.[72,73]	Exposure to OA results in morphological features of apoptosis in human lymphocytes
Llado V et al.[58]	Minerval induces apoptosis in Jurkat cells
Mechanisms involved	
Franson R et al.; Alaoui El Azher M et al.[97,98]	OA inhibits secretion and PLA_2 activity
Tappia PS et al.[99]	There is a non significant increase of PLA_2 activity in the presence of OA compared with EPA or DHA, or an enhanced activity induced by linoleic acid
Kambe T et al.[79]	OA induces a lower AA release than n-6 PUFA
Casabiell X et al.[100]	OA blocks signal transduction by interfering with PLC-receptor interaction without preventing ligand binding
Sanderson P et al.[101]	A diet rich in OA does not affect PIP_2 level in lymphocytes, but decrease the concentration of IP_3 in stimulated lymphocytes
Irvine RF et al.[102]	OA stimulates specifically the hydrolysis of membrane PI
Hwang SC et al; Sekiya F et al.[103,104]	OA activates PLC in combination with proteins as tau or AHNAK, but fails to act alone
Frohman MA et al.; Kasai T et al.; Nakashima S et al.[111-113]	PLD PIP_2-insensitive are activated by OA and drastically increased during apoptosis of Jurkat cells
Bartoli R et al.[125]	A diet rich in n-9 fats decreases mucosal arachidonate concentratrion and AA; EPA ratio as compared to n-6 diet
Chow SC et al.; Richieri GV et al.; Gamberucci A et al.[47,129,130]	OA inhibits the agonist-induced extracellular Ca^{2+} influx in T cells
Gamberucci A et al.[84]	OA discharges almost all the thapsigargin-sensitive Ca^{2+} pool
Carrillo C et al.[138]	A DAG-containing OA can activate TRPC3 and TRPC6 Ca^{2+} channels in Jurkat cells

Adaptative immune response: key role of lymphocytes

Effects of OA on lymphocyte functions

Oleic acid in T cell proliferation

Several studies have reported a potential role of MUFA-rich diets on immunomodulatory processes based on its ability to influence the proliferation of immune cells.

Verlengia et al. have reported an in vitro inhibiting effect of OA on the proliferation of Jurkat T cells and a reduction in the production of IL-2 and INF-gamma.[57] These findings are further corroborated by minerval, an OA synthetic analogue, which also inhibited proliferation of Jurkat cells.[58]

Animal studies have reported an inhibition of lymphocyte proliferation in response to a T-cell mitogen. Thus, rats fed for weeks on olive oil diets showed a consistent inhibition of spleen and lymph node lymphocyte proliferation.[59,60] Similarly, feeding cashew kernel oil, which is rich in OA, also resulted in an inhibition of rat spleen lymphocyte proliferation compared with feeding them the coconut oil diet.[61]

Olive oil contains a number of antioxidants, sterols, hydrocarbons and alcohols. In order to elucidate whether the effects reported above were due to OA or to some other component of the oil, Jeffery et al. fed rats for 6 weeks on diets containing 20% by weight of olive oil, safflower oil, or high OA sunflower oil (HOSO), using a low-fat diet containing 2.5% by weight of lipid as a control. The results showed a significantly lower mitogen-stimulated spleen lymphocyte proliferation following olive oil or HOSO feeding as compared with low-fat or safflower oil feeding, these observations indicating that the effects of olive oil feeding are most likely due to OA rather than to other components of olive oil.[62]

Moreover, the use of such oils results in variation in the levels of several fatty acids together and not only the one under investigation. In this sense, to obtain further information about the immunomodulatory effects of specific dietary fatty acids some investigators performed a controlled study in which one fatty acid was exchanged for another, without altering the levels of other fatty acids in the diet. The nine diets used differed in their contents of palmitic, oleic, linoleic and α-linolenic acids. The proliferation of spleen lymphocytes decreased as the level of OA in the diet increased.[63]

Other studies on the proliferation induced by fatty acids "per se", showed an enhanced lymphocyte proliferation, IL-2 and TNF-α production, induced by a Rice bran oil diet (rich in linoleic acid) compared to a HOSO.[64]

Studies in humans are lacking and the results available differ from the findings obtained in laboratory animals above reported. Consumption of a MUFA-rich diet did not affect the proliferative response of cells, either in whole-blood cultures or in peripheral blood mononuclear cells, to the T cell mitogen concanavalin A.[65] Such controversial results could be related to the doses tested.

The mechanisms by which OA influences T lymphocytes functions are not clear but this fatty acid seems to be involved in the regulation of cell cycle. Epithelial growth factor receptor (a critical crossroad of multiple receptor pathways which is potentially implicated in the regulation of proliferation and possibly involved in atherogenesis) could be considered a target for unsaturated fatty acids.[66] Mata et al. have demonstrated that MUFA diets produce the lowest induction of smooth-muscle-cell entry in the cell cycle when compared with other fatty acid-enriched diets although these authors did not investigate whether or not this effect influences cell division and proliferation.[67]

Oleic acid and NK cells

Natural killer (NK) cells are part of natural rather than specific immunity, since they are not activated by a specific antigen. However, these cells are a subset of lymphocytes, found mainly in the blood and spleen, and that is the reason why they are included in this section. They are derived from the bone marrow but they do not undergo thymic maturation.

Rats fed for weeks on a diet rich in olive oil resulted in a significant suppression of NK cell activity, compared with feeding a low-fat diet or diets containing hydrogenated coconut oil or safflower oil, although it was not as great as that resulting from feeding a diet containing fish oil.[68] Jeffery et al. demonstrated once again that the inhibitor effect of olive oil feeding on NK cell proliferation is most likely due to OA rather than to other components of olive oil.[62] In addition, other reports have focus on studying the single effect attributable to OA itself finding a significant negative linear relationship between the OA content of the diet and NK cell activity, suggesting that dietary OA causes diminished NK cell activity.[63]

However, as happened in lymphocytes proliferation, studies developed in middle-aged men, have given controversial results. These subjects increased their OA intake at the expense of saturated fatty acids and after a month there was a non-significant reduction in NK cell activity.[65] Once again, the differences observed between animal and human studies are likely to be due to the extreme doses added in laboratory research.

Apoptotic effects of OA in T cells

Programmed cell death or apoptosis is a physiological process to get rid of non-functional or surplus cells. With regard to T lymphocytes, this process make possible to eliminate those lymphocytes having a T cell receptor (TCR) that recognize their own antigens (self tolerance) during thymic maduration. Equally, once the attack has been neutralized, the immune system eliminates the excess of T lymphocytes activated in order to restore "the pool" of these cells. Such two pathways for the removal of T lymphocytes, are developed trough a kind of apoptosis known as "AICD" (activation-induced cell death).[69-71] Both autoimmunity, caused by recognition of self antigens, and inmunopatology, caused by a senseless presence of excessive amount of T lymphocytes activated, can be prevented by AICD.

Human lymphocytes, Jurkat (T lymphocyte) and Raji (B lymphocyte) cells, show morphological features of apoptosis after exposure to OA.[72,73] In addition, Llado et al. reported for the first time that minerval, an OA synthetic analog, also induced apoptosis in Jurkat T cells.[58] Thymocytes are also affected by PUFAs treatment, but no studies have been developed with OA.[74]

The mechanism of cell death induced by these fatty acids seems to be related to mitochondrial depolarization and ROS production or caspase 3 and 6 activities production. Moreover, evidence is presented that OA is less toxic to human lymphocytes than linoleic acid so OA may offer an immunologically less harmful alternative to linoleic acid for parenteral and enteral diets preparation.[72,73,75,76]

As previously reviewed, OA could be considered a potential immunomodulatory fat. However, the mechanisms by which this fatty acid exerts its effects are still unclear and available evidences will be described in next section.

Mechanism involved in the immunomodulatory effects of OA

The main target of unsaturated fatty acids is the membrane where they are going to be incorporated into

the phospholipids. Once incorporated, these fatty acids could affect cellular function directly or indirectly. In fact, several hypotheses concerning the mechanisms of action of unsaturated fatty acids have been postulated:

- Increase in membrane fluidity which improves membrane-protein interaction and modulates signal transduction.[77]
- Decrease in AA content in membrane phospholipids and therefore, arachidonic-derived eicosanoids, known as proinflammatory agents.[78,79]
- Improvements of oxidant status as MUFAs are less sensitive to lipid peroxidation and ROS production.[80]
- Modulation of intracellular pathways that play a central role in cell activation.[81-85]
- Modulation of gene expression involved in cytokine production.[57]

Lipid signaling modulation

Membrane phospholipids are involved in the signal transduction pathways started out of the cell. Dietary unsaturated fatty acids or cells treatment with fatty acids, leads to their esterification in sn-2 position of membrane phospholipids. Once incorporated in the phospholipids, they can be hydrolyzed by several phospholipases. Phospholipases A_1 and A_2 (PLA_1 and PLA_2) lead to the release of those fatty acids present in sn-1 and sn-2 position of the glycerol, respectively, and to the subsequent lysophospholipides generation. Phospholipase C (PLC) hydrolyzes PIP_2 releasing also two second messengers: DAG and IP_3. Phospholipase D (PLD) acts mainly on phosphatidilcoline (PC) and leads to the production of phosphatidic acid (PA), which is rapidly hydrolyzed to form DAG. Additionally, PA can also activate PLC and increase the affinity of the enzyme for the subtract.[86,87] As PA is the result of the hydrolysis of PC by PLD, the PLD activation can also lead to the activation of PLC.

Stearic acid and palmitic acid are almost exclusively located at the sn-1 position of the different glycerophospholipids, with the majority of OA and other unsaturated fatty acids found at the sn-2 position.[88,89] Thus, n-9 MUFA taken by the diet will result in its incorporation at sn-2 position of membrane phospholipids instead of AA. Depending on the action of different phospholipases, it will affect intracellular signaling pathways both as a free OA (under the action of PLA_2)[90,91] or as a DAG-containing OA (under the action of PLC and PLD).[92]

Phospholipase A2

Phospholipase A_2 enzymes catalyze the hydrolysis of ester bonds at the sn-2 position of membrane phospholipids and simultaneously release fatty acids, such as AA and lysophospholipids.[93,94] The hydrolysis of phospholipids by PLA_2 is a key phase in the regulation of inflammatory processes as AA release leads to the production of eicosanoids.[95] Stimulus activation of PLA_2 can release OA as well, a non-proinflammatory-mediator.[96]

Several studies have demonstrated that unsaturated fatty acids, including OA, can inhibit both secretion and activity of PLA_2[97,98] results suggesting anti-inflammatory roles for OA during the inflammatory reaction, as AA release and thus subsequent products from its metabolism are inhibited. By contrast, other studies claim a non-significant enhancement of PLA_2 activity in the presence of OA, compared with a suppression effect of EPA or docosahexaenoic acid (DHA) or an enhanced activity induced by linoleic acid.[99]

Furthermore, unsaturated fatty acids are reported to be capable of regulating the enzymatic system involved in their own release. In this sense, Kambe et al. have reported that cells incubated with exogenous unsaturated fatty acids in the presence of IL-1 and serum, showed a significant increase in AA release. OA proved to be the one which induced AA release in a lower amount; AA and linoleic acid were more potent.[79]

Phospholipase C

Phosphatidylinositol-specific PLC (PI-PLC) plays an important role in cell signal transduction. PLC can hydrolyse phosphatidilinositol 4,5 biphosphate (PIP_2) into two second messengers: IP_3 and DAG. These messengers then promote the release of Ca^{2+} from intracellular stores, and the activation of PKC, respectively.

A number of studies have reported the effect of fatty acids on PLC activity. Unsaturated fatty acids, such as OA, but not saturated ones, block signal transduction by interfering with receptor-PLC or PLC-substrate interaction without preventing ligand binding.[100] Sanderson and Calder developed a study with rats feeding them on diets with different oils and they showed that the level of PIP_2 in spleen lymphocytes was unaffected by diet. However, an olive oil diet (as well as a fish oil one) significantly decreased the concentration of IP_3 in stimulated lymphocytes. However, in stimulated lymphocytes, the phosphorylation state of the enzyme, as well as that of a range of other proteins, was decreased following feeding on olive oil diets (and fish oil ones).[101]

By contrast, some researchers showed that addition of OA to rat microsomal fractions stimulates specifically the hydrolysis of membrane PI.[102] However, these unsaturated fatty acids do not directly affect PIP_2-hydrolysis activities of various PLC isozymes. Hwang et al. demonstrated that unsaturated fatty acids, such as OA, enhance the activation of PLC by tau, a protein known to activate phospholipase.[103] In addition, AHNAK, another protein, in combination with unsaturated fatty acids, such as OA, can activate PLC. These authors did not find an activator effect of the fatty acids alone either.[104]

Other researchers have focused on studying the effect of the incorporation of different fatty acids in inositol lipids. These results showed that fatty acids induced modifications in PLC activity. PIP_2-PLC activities reached a maximum when inositol lipids containing OA became more abundant than normal.[105]

Phospholipase D

Phospholipase D is widely distributed in mammalian cells and is implicated in a variety of physiological processes that reveal it to be a member of the signal transducing phospholipases. PLD is an enzyme which is located in the plasma membrane, mainly in caveoles, membrane specialized domains rich in sphingolipids and cholesterol "lipid rafts".[106] It catalyzes the hydrolysis of PC to form PA, releasing the soluble choline headgroup into the cytosol. PA is extremely short lived and is rapidly hydrolysed by the enzyme PA phosphohydrolase to form DAG.

Two families of PLD can be distinguished depending on their sensitivity to PIP_2. Some PLD can be activated only in the presence of such a lipid (PLD PIP_2-sensitives), while others do not need it (PLD-PIP_2-insensitives).

Whereas PLD PIP_2-sensitives are activated by several proteins,[107-110] the PLD PIP_2-insensitives are activated by OA[111] and therefore known as PLD-oleate dependent.[112] This type of PLD activity is drastically increased during apoptosis of Jurkat T cells. In fact, PLD activation in lymphocytes T is related to antiproliferative and apoptotic signals. Such results suggest the possibility that PLD plays roles in differentiation, survival and apoptosis in mammalian cells.[112,113]

These findings could be of physiological relevance when studying the mechanisms of OA acid induced apoptosis in T cells.

Diacylglycerol

A diglyceride, or a diacylglycerol, is a glyceride consisting of two fatty acid chains covalently bonded to a glycerol molecule through ester linkages. In mammalian cells there are, at least, 50 structurally distinct molecular species of sn-1,2-diacylglycerol,[114,115] whose fatty acyl groups can be polyunsaturated, monounsaturated or saturated. When cells are stimulated by appropriate agonists, PIP_2 hydrolysis catalysed by PIP_2-PLC becomes an important source of new DAG that are able to activate PKC. Cell stimulation also commonly activates PLD-catalysed PC hydrolysis. The PA generated by PLD is thought to be an intracellular signal whose action also results in DAG production.

Phosphatidilinositol 4,5 biphosphate and PC (the precursors of DAG) typically have very different fatty-acyl complements and their hydrolysis yields very different DAG. Inositol lipids, which make up 5-10% of the total phospholipid content of most mammalian cells, are mainly polyunsaturated: 30-80% of total phosphoinositide is typically the sn-1-stearoyl-2-arachidonyl species.[114-118] PC is much more abundant, making up around 30-50% of total mammalian cell phospholipids. In many cells, PC predominantly contains saturated and mono-unsaturated fatty acids; relatively few PC species are polyunsaturated.[114-118]

Dyacylglycerol production in stimulated cells is often biphasic: there is an initial rapid rise in DAG concentration and then, a slower accumulation that can be sustained for an hour or more. Polyunsaturated DAGs (polyDAGs) —with a saturated or mono-unsaturated 1-acyl group and a polyunsaturated 2-acyl group— predominate during the initial phase; however, they are largely restricted to a few polyDAG species, notably 1-stearoyl-2-arachidonyl-DAG. During the sustained phase, the concentrations of a broad range of monounsaturated DAGs (monoDAGs) and saturated DAGs (satDAGs) rise, and these are accompanied by smaller amounts of polyDAGs. The initial polyDAGs are mainly products of PIP_2 hydrolysis, whereas the mono/satDAGs generated in the sustained phase are derived predominantly (through dephosphorylation of PLD generated PA) from PC.[114,115,119]

Moreover, fatty acid composition of membrane phospholipids can be further influenced by the diet. Previous studies have shown that in T-cells exposed to an OA-rich medium, the total monounsaturated fatty acyl content was increased by 130% in PI, 100% in phosphatidylserine (PS), 160% in phosphatidylcholine and 180% in phosphatidylethanolamine (PE).[120] Other researchers showed that palmitic and oleic acids were found preferentially incorporated into PC and the majority of the highly unsaturated fatty acid, AA, was incorporated into both PE and the PI-PS group.[121] In any case, during the dietary intake of n-9 fatty acids, the cells will be enriched with this fatty acid and a cell activation via PLC- or PLD-pathway may give rise to the production of DAG in the conjugated form of 1-stearoyl-2-oleyl-sn-glycerol (SOG), as it has been previously reported in human Jurkat T-cells.[122]

Several reports have proposed that the fatty acids that make up the phosphoinositides, function as intracellular modulators of the activity of certain enzymes.[105] The best characterized function of receptor-stimulated DAG production is activation of PKC, although not all DAG species are capable of activating this enzyme.[123] In this sense, Madani et al. have demonstrated differences in modulating the activity of several isoforms of PKC by two polyDAG: SEG (DAG-containing EPA) and SDG (DAG-containing DHA).[124] Additionally, some channels in the membrane that can be directly activated by DAG have been recently identified (see below).

Eicosanoids production

Oleic acid could exert its antiinflamatory effect by influencing AA metabolism. This PUFA is in turn the

precursor of the eicosanoids proinflamatory agents. Thus, competitive substitution of membrane arachidonate by n-9 fats could be associated with modifications in the rate of arachidonic-derived metabolites release, suggesting that as a mechanism for the OA induced-immune modulatory pathways. In this sense, evidences show that the n-9 diet significantly decreased both mucosal arachidonate concentrations and AA:EPA ratio as compared with the n-6 diet, which may in part account for the observed beneficial effect of olive oil.[125]

Signaling pathways modulated by oleic acid

Fatty acids can modulate several signaling pathways, from the antigen presentation to the T cell proliferation. In the present review we will focus on the role of OA in Ca^{2+} signals.

Ca^{2+} signaling

Most of the studies available in immunocompetent cells evaluate the effect of polyunsaturated n-6 and n-3 on Ca^{2+} signaling. Although OA has been reported to alter C^{a2+} homeostasis in several cell lines,[126,127] little is known about its effects concerning T cells.

Oleic acid has been demonstrated to induce increases in $[Ca^{2+}]i$ in several cell lines.[91,126-128] By contrast, other studies have demonstrated that OA inhibit the agonist-induced extracellular Ca^{2+} influx in T cells,[47,129,130] and other cell types.[131,132] With regards to the mechanisms behind these alterations in Ca^{2+} homeostasis, Gamberuci et al. have demonstrated that OA discharges almost all the thapsigargin-sensitive Ca^{2+} pool. In addition, neither pretreatment of the cell with the G-protein interfering agent pertussis toxin nor with a phospholipase inhibitor prevents the induction of Ca^{2+} mobilization by OA.[84] Researching further the mechanisms by which such Ca^{2+}-mobilizing effects occur, these authors suggest that an increased Ca^{2+} efflux from thapsigargine-sensitive pool is more likely to occur than an inhibition of active Ca^{2+} transport into the same pool. However, although these data supported those reported by Chow and Jondal for PUFAs, these researchers did not find similar results concerning OA.[46]

In addition, Ca^{2+} channels such as TRPC3, TRPC6 and TRPC7 have been reported to be activated by the exogenous addition of DAG.[133-136] Aires et al. have studied the effect of a DAG-containing DHA in the activation of TRPC channels.[137] Results from our group have recently reported a role of a DAG containing OA in the activation of TRPC6 and TRPC3 channels in human Jurkat T cells.[138]

Other mechanisms

One emerging view is that the potential mechanism by which PUFAs influence immune cell function could be related to its ability to modulate lipid rafts. Lipid rafts are postulated to be specialized lipid microdomains rich in sphingolipids and cholesterol. They are involved in serving as platforms for cellular signaling events.[139-142] For instance, the ER Ca^{2+} sensor STIM1 (Stromal Interaction Molecule 1) has been reported to interact with Orai and TRPC in these specific parts of the membrane, leading to the extracellular Ca^{2+} entry.[143,144] It has been recently described that the disruption of lipid rafts avoids the interaction between STIM1 and Orai1.[145,146]

The supposed stability of lipid rafts is attributed to the favorable interaction between the amide of the sphingosine backbone and the hydroxyl of an adjacent sphingolipid as well as hydrogen bonding between the 3-OH of cholesterol and the sphingosine amide. Saturated acyl chains are thought to promote the formation of rafts since they are more extended than unsaturated chains and packed well amongst themselves and with cholesterol. Previous reports have shown that PUFA can modify the composition of lipid rafts.[147-151] In this sense, several researchers have demonstrated that T cells submitted to PUFA treatments, results in an inhibition of different signal transduction pathways.[152-156] However, most of the studies in literature involved PUFA and OA disruption of lipid rafts is still unclear.

Oleic acid in clinical nutrition

As we have reported above, diets containing a high amount of olive oil in experimental animals, produce a suppression of lymphocyte proliferation, an inhibition of cytokine production and a reduction in NK cell activity.[57,59,60,68] Despite these alterations in immune functions, it has been reported that olive oil-rich diets are not as immunosuppressive as fish oil diets. An important aspect in inmunonutrition is focused on the relationship between fats, the immune system and host resistance to infection, particularly when these nutrients are supplied to patients at risk of sepsis. Different studies have determined that olive oil-rich diets do not impair the host resistance to infection.[157,158] Therefore, olive oil constitutes a suitable fat that may be applied in clinical nutrition and administered to critically ill patients.

The current knowledge of these aspects and the beneficial effect attributed to an olive oil-rich parenteral emulsion (Clinoleic) have been recently reported elsewhere.[157-160]

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