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Altered Inflammatory Mediators in Fibromyalgia

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Abstract

Fibromyalgia (FM) is a syndrome characterized by widespread chronic pain, tenderness, stiffness, fatigue, and sleep and mood disturbances. Current evidences suggest that inflammatory mediators may have an important role in the pathogenesis of fibromyalgia. Every day new evidences emerging of the role of the immune system and the inflammatory process in the pathophysiology of this disease. Thus, the aim of this work has been review that altered inductors, inflammatory mediators and effectors have been reported in fibromyalgia, and its relationship to disease's symptoms. If in fibromyalgia underlies widespread disruption of most characteristic components on the inflammatory ´s process (endogenous inductors, mediators and effectors) is logical to think that fibromyalgia is producing a sustained inflammatory response in time, unregulated, responsible for the disease´s symptoms.

Keywords: Fibromyalgia; Inflammation; HMGB1; Age; Inflammasome; Cytokines; Chemokines; Pentraxins; Lipid mediators; Monocytes; Neutrophils

Fibromyalgia

Fibromyalgia (FM) is a chronic disease characterized by a widespread pain, tenderness, stiffness, fatigue, and sleep and mood disturbances. The prevalence of FM in the world ranges between 0.7% and 3.2%. It is more prevalent on women than men with a ratio women/men 20:1 (4.2% prevalence on women compared with 0.2% on men) [1] and it can develop at any age [2].

The exact cause of fibromyalgia is unknown, but it has been proposed that the FM is a stress- related disorder that may involve a dysfunction of the hypothalamic-pituitary-adrenal axis (HPA) [3,4]. Thus, adverse life events, such as emotional, physical and sexual abuse on children and adults have been suggested to contribute to the development of FM [5-7].

Risk factors FM have been the subject of much debate in recent research. There is no doubt that the experience of an abusive relationship, maintained over time, is a stressful situation that could be related to the presence of FM [8,9].

Like all physiological systems, the immune system is under the control of the neuro-endocrine system and interacts and works in concert with other regulatory body systems, thus immune cells respond to neuroendocrine signals through specific receptors for hormones, neurotransmitters and neuropeptides. Therefore, dysregulation of the hypothalamic-pituitary-adrenal axis by chronic stress situations necessarily lead to a dysregulation of the immune function. Thus, inflammation is a characteristic process of the immune system, considered a defense response that is induced by infection or trauma. However, inflammation may also be induced by stress and tissue dysfunction in the absence of infection, there is a close relationship between body homeostasis, stress response and inflammation, since inflammation has both a component response to stress as a component defense response.

Inflammation and Fibromyalgia

In 2001, Wallace et al. [10] were the first to suggest that inflammatory mediators, such as cytokines, could play a role in fibromyalgia. Prior results to Wallace´s et al. hypothesis [10] had found high levels of inflammatory Substance P neurotransmitter and calcitonin gene-related peptide (CGRP) in the spinal fluid from FM patients [1-13]. Platelet serotonin levels were also abnormal [13]. Furthermore, in FM patients, the pain intensity level was related to the level of arginine in the spinal fluid, which is the precursor of inflammatory mediator nitric oxide [14]. So, when Wallace et al. [10] found increased levels of IL-8, IL-6 and IL-1RA in FM patients and their release was stimulated by Substance P, they concluded that because IL-8 promotes sympathetic pain and IL-6 induces hypersensitivity to pain, fatigue and depression, both inflammatory cytokines play a role in producing FM symptoms.

Later Omoigui [15] proposed the unifying theory of pain for which the source of all pain is inflammation and inflammatory response. The activation of pain receptors, transmission and modulation of pain signals, neuroplasticity and central sensitivity are all "one continuum" of inflammation and inflammatory response, he also suggested that the pain suffered by women with fibromyalgia it was as a result of neurogenic inflammation present in this disease [16].

Each day, there are more evidence about the immune system´s role in the pathophysiology of FM, either as an adaptive response to stress situations, maintained over time, and as a consequence of a dysfunction of the immune cells due to chronic stress situations which the body was not able to adapt. Many studies have found altered inflammatory mediators in FM which could be inducing the disease´s characteristic symptoms such as pain, fatigue, depression, sleep dysregulation, etc.

On the other hand, under long lasting extreme conditions of cellular stress, when apoptosis fails, it usually happens necrosis. An important consequence of necrotic cell death is the loss of membrane integrity, allowing the output of the cell´s intracellular material. Among the released material are the DAMPs ("danger-associated molecular patterns") which are prototypical molecules released by necrotic cells, included among them are the chromatin-associated-protein high-mobility group box-1 (HMGB-1) [17,18], heat shock protein (HSPs) [19] and purine metabolites such as ATP [20] and uric acid [21], all them activate the inflammatory response [22].

Inflammation is an adaptive response that is triggered by stimulation and/or harmful conditions, such as, and for example infection or tissue trauma. Although the adaptive response in regard to acute inflammation is well known, the localized chronic inflammation is partially known and the causes and mechanisms of systemic chronic inflammation are much less known, which occurs in a variety of diseases including diabetes type 2 and cardiovascular diseases [23].

Functional Components of Inflammation

The inflammatory response is coordinated by a large range of mediators that form a complex regulatory network. In order to study this complex network is convenient to divide these signals into functional categories thus distinguish between inflammation´s inductors, mediators and effectors. The inductors are signals that initiate the inflammatory response and may be endogenous or exogenous. These specialized inductors activate sensors which then elicit the production of a specific set of inflammatory mediators. Mediators, in turn, alter the functional state of tissues and organs (which are the effectors of inflammation) in a way it can adapt to the conditions set by the particular inducer of inflammation [23].

Therefore, the aim of this task is to review that inductors, inflammatory mediators and effectors have been reported altered in fibromyalgia, so that, if in fibromyalgia underlies widespread disruption of all characteristic components on the inflammatory´s process (endogenous inductors, mediators and effectors) is logical to think that fibromyalgia is producing a sustained inflammatory response in time, unregulated, responsible for the disease´s symptoms.

Endogenous Inflammatory Inducers Altered in Fibromyalgia

Endogenous inducers of inflammation are signals that initiate the inflammatory response and are produced by damaged or malfunctioning stressed tissues. These inflammatory endogenous inductors belong mostly to DAMPs released when rupture of the cells by necrosis. Thus, their high concentration presence on fibromyalgia could indicate that in this disease there are malfunctioning ontissues or cells, which are stressed or even damage, developing an inflammatory response as a situational response with subsequent induction of pain.

For example, during necrotic cell death (but not with apoptosis) the integrity of the plasma membrane is broken, resulting in the release of certain cellular constituents that act mostly as inflammation inducers, pointing to the organism that there is altered tissue and cell homeostasis, triggering an adaptive response such as inflammation. These components include HMGB1 (high- mobility group box-1), ROS (reactive oxygen species), AGEs (advanced glycation end products), uric acid, ATP, etc. In recent years, some studies have reported alterations of these molecules in patients with fibromyalgia.

HMGB1 (High-Mobility Group Box-1 Protein)

HMGB1 is one of the first identified members of DAMPs, molecules that emerged from Matzinger´s ideas [24] which the innate immune system detects and reacts to "danger" through the release of host-derived mediators.

The DAMPs are generally nuclear and cytosolic endogenous proteins running well-defined intracellular roles in the absence of cellular stress. When they are released extracellularly after tissue damage or trauma, these molecules promote innate and adaptive immune responses and do not maintain their previous intracellular activities. The HMGB1 starts and perpetuates immune responses during infection and sterile inflammation, like a typical alarmin molecule and DAMPs [25].

So, the extracellular HMGB1 levels were found increased in patients with FM (n=29) compared with healthy people (n=29) and positively correlated with the FIQ score of these patients [26]. This molecule also acts as a proinflammatory cytokine that through TLR receptors (Toll-like receptor) and RAGE (receptor of advanded glycation end-products) enhances the production of TNF-α, IL-6, IL-1 proinflammatory cytokines, as we are going to indicate further on this work, and they are increased in fibromyalgia. Indeed, HMGB1 is a key factor in the pathogenesis of a wide variety of inflammatory conditions and was also identified as a mediator of neuroinflammation.

Experimental studies suggest that the HMGB1 is released both spinally and peripherally on models of pathological pain and can lead the nociceptive signaling *via* actions on sensory neurons and / or glia and immune cells. The HMGB1 has pronociceptive properties in both the central and peripheral nervous system [27]. In addition, there are emerging evidences that TLR- and RAGE-induced signaling pathway contributes to the painful hypersensitivity. Because the HMGB1 is a multireceptor endogenous ligand for these receptors and play a central role in chronic pain, serves as a critical link between the sterile inflammation, and the pattern recognition receptors (PRR) and also for the activation of the sensory nervous system [27].

Until now, we have not found more studies on the relationship of this inflammatory inductor and fibromyalgia, so, we understand that it would be really necessary to deepen on the role of HMGB1 in FM pathophysiology.

ROS (Reactive Oxygen Species)

Eukaryotic cells use the oxidation of nutrients as an energy source to obtain ATP. In the complete glucose oxidation process by cells are involved various metabolic pathways (glycolysis, Krebs cycle and the electron transport chain (ETC)) and some cellular compartments (cytoplasm, membrane and mitochondrial matrix). Under physiological conditions, the enzymes of the mitochondrial respiratory chain, as a result of metabolism, are responsible for a constant endogenous production of free radicals (such as reactive oxygen intermediates (ROI) and reactive nitrogen species (RNS)) [28]. The main source of ROS generation is the electron transport chain (ETC) in the mitochondria. However, there are also non-mitochondrial sources of reactive oxygen molecules and free radicals that include, among them, the respiratory burst of phagocytic cells.

However, biological systems developed the ability to detoxify the ROS and RNS chemically active. These antioxidant mechanisms include non-enzymatic complex systems such as glutathione (GSH),

vitamin A, vitamin C and vitamin E, as well as, enzymes as catalase, superoxide dismutase (SOD) and several peroxidases. When an imbalance is produced between free radicals and antioxidant defenses due to excessive and uncontrolled generation of ROS then oxidative stress occurs [29,30]. Both reactive oxygen species and oxidative stress are inducers, therefore, of the inflammatory response.

The ROS, especially derived from mitochondria, stimulate activation of the signaling molecules mediators as the transcription factor Kappa-Beta (NFkβ) [31] which upregulates the production of proinflammatory cytokines such as TNF-α and IL-1β [32] and other mediators such as iNOS or cyclooxygenase-2 (COX-2) [33]. In addition, ROS may damage cell lipids, lipid peroxidation products and aldehydes derived from lipid as malondialdehyde (MDA), 4-hydroxy-2- nonenal (HNE) and acrolein, which are involved in numerous inflammatory diseases induced by oxidative stress with detrimental effects [34]. Thus, ROS and inflammation were identified in the pathogenesis and development of a large number of diseases, also including fibromyalgia [10,30,35]. Previous studies reported the presence of oxidative stress in patients with fibromyalgia but results have been inconsistent, as to, whether reported abnormalities of oxidative stress are the cause of fibromyalgia, however it seems clear that oxidative stress and free radicals are involved in the pathophysiology of this disease [36].

Elsinger et al. [37] investigated that the level of malondialdehyde (MDA), a molecule which reflects the degree of lipidic peroxidation in blood plasma was not changing in patients with FM but Bagis et al. [38] reported higher levels of MDA and lower levels of superoxide dismutase (SOD) in patients with FM. Ozgocmen et al. [39] also found increased levels of MDA and lower levels of nitrite in patients with FM. Sendur et al. [40] demonstrated that glutathione peroxidase (GPx) and catalase were significantly lower in patients with FM, implying the importance of these antioxidants on FM´s pathophysiology and other neurological disorders [41]. Altindag and Celik [42] reported increased lipid peroxidation and carbonylated proteins and increases on total peroxide´s level in FM patients compared to healthy controls. Cipollone et al. [43] found a significant correlation between fatigue and increased oxidative stress and decreased antioxidant defense. Richard and Kahn [44] suggested that oxidative stress is associated not only with fatigue but also with pain or sensitive points in patients with FM. Lamb et al. [45] found increased mitochondrial ROS production in FM patients and Meeus et al. [46] also found a higher concentration of total ROS in these patients. Also, Akkuş et al. [47] and Nazıroğlu et al. [48] found a higher level of lipidic peroxidation in patients with FM. Meanwhile, Fatima et al. [49] found higher concentrations of MDA in patients with FM, agreeing results with those reported by Sanchez-Dominguez et al. [35] who also found high concentration of MDA in patients with FM. More recently, Fatima et al. [50] found levels of lipid peroxides (LPO) and carbonyls protein significantly higher in FM patients than in controls. Moreover, these results also showed that increased oxidative stress parameters are strongly associated with severity of FM since a significant positive correlation was also found between carbonyls protein and FIQ in the patient group than in the control group.

Studying the role of antioxidant defenses in FM patients, it was found lower concentrations of glutathione [48], CoQ10 [45], superoxide dismutase [39,40,42,49,51], vitamin A and vitamin E [47,48], as well as less concentrations of sPhA2 (secretory phospholipase A2) [52]. Fatima et al. [49] also indicated that women with FM are exposed to oxidative stress, since the activity of catalase

enzymes, glutathione reductase (GR) and glutathione peroxidase (GPx) were significantly lower in patients with FM than in controls.

Most research has been oriented to the plasma or serum of patients as a study model, creating the need of using cell models, meaning, the place where the machinery of ROS production is activated and controlled. Regarding to the superoxide anion (O^{2-}) as one of the free radicals which comes the oxygen as part of the ROS, was not found increased in neutrophils of FM patients [53,54]. However, it has been reported high levels of superoxide anion of mitochondrial origin (O^{2-}) observed in mononuclear peripheral blood cells in patients with FM [55].

In this same model, FM patients had low levels of CoQ10, an element of vital importance on the mitochondrial respiratory chain which primary mission is transporting electrons from complexes I and II to III, in addition to regulate the coupling of proteins, the mitochondrial transition pore and beta-oxidation of fatty acids and is an important antioxidant membrane, therefore, the deficiency of itself induces in the cells a decreased activity on electrons transport chain, also reduces the expression of mitochondrial proteins involved in ATP generation, decreases the mitochondrial membrane potential and increases ROS production [55]. So, this CoQ10 deficiency causes a clear cellular stress, which results in an increased oxidative stress.

It is known that ROS are involved in the etiology of one of the most important symptoms of fibromyalgia: the pain [56]. The superoxide radical plays an important role on the development of pain; on the one hand by the peripheral and central sensitization of the nervous system by inducing an altered nociception, and secondly by activating various cytokines such as TNF-α, IL-1β, IL-6, like the inflammatory inducer, HMGB1. It is possible that oxidative damage interferes on tissues by decreasing nociceptors locally, causing a decrease in pain threshold [57], which could explain the decrease in pain threshold on FM patients.

Thus ROS have been associated with an increased peroxidation of lipids, resulting in disruption of cellular structures and cellular functions, generating tissue and cells stress also acting as endogenous inducers of inflammation not only on FM but even in other autoimmune disorders. In addition, ROS and pro-oxidant conditions also favor the generation of AGEs (advanced glycation end products) by the cells subjected to oxidative stress [58].

AGEs (Advanced Glycation End-Products)

Advanced glycation end-products (AGEs) are endogenously formed when the carbonyl groups of reduced sugars react non-enzymatically with the amino groups free of proteins. AGEs are generated *in vivo* as a normal consequence of metabolism, but their formation is also accelerated under conditions of hyperglycemia, hyperlipidemia and increased oxidative stress [23,58].

AGEs excessive accumulation results on significant cell dysfunction due to inhibition of cell- cell communication, altering the protein structure and interfering with lipid accumulation in the arterial wall [59]. Interaction of AGEs with the receptor for AGEs (RAGE) activates nuclear factor kappa beta (NFkβ), firing oxidative stress, thrombogenesis, vascular inflammation and pathological angiogenesis [58,60]. These molecules have similar effects to HMGB1 and also bind with RAGE. Recently, AGEs have been implicated with diabetes type 2 pathogenesis, contributing to the development of insulin resistance

and to low grade inflammation which is known of preceding to this condition [58,61,62].

The AGEs was found elevated in inflammatory rheumatic diseases such as rheumatoid arthritis, where it was found high levels of pentosidine. AGE pentosidine formation, a fluorescent cross-linked structure of lysine and arginine, requires both glycation and oxidation, being closely related to oxidative stress [58,63,64]. These high concentrations of pentosidine found in serum, urine and cartilage in rheumatoid arthritis [58,65-67] with a significant correlation between serum pentosidine and disease activity levels. Thus, in 2002, Hein and Franke [68] found for the first time, higher levels of pentosidine AGE in FM patients (n=41) when compared with the levels of healthy people groups (n=56). Subsequently, Rüster et al. [69] reported that other AGE, N (epsilon) - carboxymethyllisine (CML), was increased in the blood of patients with FM (n=41) with respect to the levels of healthy people (n=81). The AGE modification of proteins, accelerated by a hypothesized tissue hypoxia in the muscles, tendons and related skin tissues of FM patients, may also induce cell activation, for example, macrophages, fibroblasts or peripheral nerve cells *via* the binding of AGE structures to their cellular receptor RAGE, followed by a prolonged activation of NFkβ. Unlike unmodified proteins, AGE-modified proteins are able to stimulate the secretion of pro-inflammatory cytokines in RAGE-bearing cells. Pro-inflammatory cytokines in turn may contribute to pain generation and perpetuation. Both mechanisms, caused by the AGE modification of proteins, ultimately interact with one another, and in a possible circulus vitiosus, the clinical symptomatology swells leading to the spreading and increased levels of pain as often seen in patients with FM [68].

Other Inflammation Inductors

There are other inflammation´s endogenous inductors, described in the scientific literature [23] such as, for example, uric acid or adenosine-triphosphate (ATP), which are released into the extracellular medium when the loss of cellular membrane integrity occurs and acting in such cases as inflammatory inductors.

Regarding to uric acid, we have only found, in the review we conducted, two studies about the role of this molecule in fibromyalgia, however the results are not consistent because Bonaccorso et al. [70] found no differences in plasma levels of uric acid in FM patients and healthy people, while Aarflot et al. [71] found increased systemic concentration of this molecule in FM patients.

On the other hand, changes in ATP in FM patients have been detected in a greater number of studies; however, these have been addressed through the prism of possible metabolic dysfunction present in muscle cells of patients with FM, assessing in most of them the intracellular concentration of ATP. So, it was found a lower intracellular concentration of ATP in muscle cells [46,72-75] and in neuronal cells [46] and peripheral blood mononuclear cells [76,77] of FM patients. We only found a study which evaluated plasma levels of ATP in patients with FM [78] this study reported lower ATP levels in the plasma of FM patients with respect to the healthy people group.

Inflammatory Mediators in Fibromyalgia

Inflammatory inducers trigger the production of numerous inflammatory mediators, which in turn alter the functionality of many cells of the tissues and organs. These mediators can be derived from plasma proteins or secreted by cells [79,80]. Cellular mediators can be produced by specialized cells or leucocytes present on local tissues. Others are preformed and circulate as inactive precursors in plasma. The plasma concentration of these mediators can be increased markedly as a result of increased secretion of precursors by hepatocytes during the acute phase response. And other mediators are produced directly in response to appropriate stimulation by inducing inflammation. In this task we focus on reviewing the alteration of inflammatory mediators, which have been reported in the FM, such as inflammasome, cytokines, chemokines, acute phase proteins (as pentraxins) and lipid mediators. We will not get into reviewing vasoactive amines (serotonin) and vasoactive peptides (Substance P) because although they function as inflammatory mediators their most important function is within the nervous system.

The inflammasomes are known for their role in the maturation and secretion of proinflammatory cytokines such as IL-1β and IL-18, but can also induce regulated cell death. Until recently, programmed cell death was conceived as a simple set of molecular pathways. Now, we know that the several and different set of mechanisms that induce cell death lead to different cell death processes. In one of them - apoptosis-, dead cells have little affect to others. In contrast, necrotic programmed cell death causes release of immunostimulatory intracellular components after cell membrane rupture [81].

The primary role of inflammasome and its products seems to be as part of the innate immune system. The NLRP3 inflammasome is also proficient on detecting signs (non-microbial) of endogenous damage (DAMPs) of damaged cells [22].

Inflammasome in FM

ROS have proved to be an important inflammation activator mediated by inflammasome [82,83]. The inflammasomes are multiproteic platforms which are organized in the cytosol to detect pathogens and cellular stress. They are signaling platforms which are assembled in response to molecules from to pathogen-associated (PAMP) and damage-associated (DAMP) molecular patterns and irritants [81].

The NLRP3 inflammasome (NOD-like receptor family, Pyrin domain containing 3) is a multiprotein, large cytoplasmic complex (>700kDa), composed by a specific member of the NOD-like receptor protein (NLRP)subfamily, the adaptor protein called apoptosis - associated spech-like protein containing a CARD (ASC) and procaspase-1, which are preferentially expressed by macrophages in adipose tissue [84,85].

The NLRP3 inflammasome is a proteolytic caspase-1 activating platform. Caspase-1 is autocatalytically cleaved to its active form. Caspase-1 does not play a major role in apoptosis. Instead, once activated, caspase-1, as far as we are currently aware, breaks the proforms of two potent proinflammatory cytokines on cytoplasm, the IL-1β and IL-18. Consequently, it has two main effects: activate these two cytokines and in this mature form, these cytokines can be released out of the cell.

The active form of caspase-1 also has the ability to induce the release of IL-1α and HMGB-1, and initiate a lytic form of cell death called pyroptosis, a way of inflammatory lytic cell death [85-88]. The pyroptosis has been implicated in the secretion of IL-18 and IL-1β and intracellular alarmins. Therefore, inflammasomes have been increasingly implicated in the induction of additional modes of regulated cell death, such as pyronecrosis and necrosis [89].

Cordero et al. [83] found increased gene expression of NLRP3 and caspase-1, the protein expression levels of NLRP3 and also found increased the cleavage of caspase-1 in BMCs (blood mononuclear cells) from FM patients, suggesting that there is a inflammasome activation in patients with FM. Interestingly, it was described that oxidized mitochondrial DNA (mitDNA) is a powerful activator of NLRP3 inflammasome [90]. In addition, other molecules involved in the activation of inflammation and inflammasome such as HMGB-1 [26], IL-1β [83], IL-18 [54,83,91] and reactive oxygen species [45,46,91] are increased in fibromyalgia. Inhibition of complex I or II of the mitochondrial respiratory chain induces ROS production and activation of the NLRP3 inflammasome [82]. In blood mononuclear cells of patients with FM, Cordero et al. [83] detected a dysfunction of the respiratory chain, CoQ10 deficiency and an increased mitochondrial ROS production along with a increased level of OGG-1 (an oxidized DNA marker), both are activators of inflammasome and inflammation [82,92].

Finally indicate that the inflammasome is emerging as a sensor of metabolic damage and stress. Thus, it has been implicated in the development of diseases such as gout, diabetes type 2, obesity-induced insulin resistance. FM patients have shown increased activation inflammasome in BMCs and increased serum levels of proinflammatory cytokines, such as IL-18 and IL-1β.

At the moment the work of Cordero et al. [83] was the only one to describe inflammasome activation in patients with fibromyalgia and its association with increased levels of inflammatory cytokines.

Cytokines in FM

Cytokines are non-structural small proteins with molecular weight ranging from 8-40000kDa, which act as messengers of the immune system and inflammatory mediators, also acting on the central nervous system. So, cytokines play a much wider role, not only in the physiology of the immune system but also in almost all organs and systems.

Cytokines are regulators of host responses to infection, immune response, inflammation and trauma. Some cytokines act to promote inflammation (pro-inflammatory) while others serve to reduce inflammation and promote healing (anti-inflammatory). Generally, pro-inflammatory cytokines are mediators of inflammatory pain, while anti-inflammatory tend to block it.

Although there is much speculation about the etiology of FM, one of the main theories is that cytokines may play a role both in the etiology of the disease and the intensity of the main symptoms [93,94]. Although the results in this regard are conflictive, increasingly studies (reviewed below) indicate that in fibromyalgia there is a generalized increase in pro-inflammatory cytokines that may be due, as we mentioned above, the high presence of inflammatory endogenous inducers and NLRP3 inflammasome activation.

Then and regarding to the role of TNF-α (pro-inflammatory cytokine) on FM the results are conflictive since studies found no differences in plasma concentrations between fibromyalgia patients and healthy people [10,95-98], those which reported an increase in TNF-α plasma concentration on patients with FM [35,83,99-103], having also published works in which a drop is detected in TNF-α concentration on FM patients compared to the healthy people control group [104-107]. However, no differences were found in concentrations of TNF-α in skin of people with FM [108] or

in muscle [109]. Although a recent study conducted by Sanchez-Dominguez et al. [35] have detected higher concentrations of TNF-αin saliva and in the skin of FM patients when compared to healthy women.

Regarding the interleukin-1β (IL-1β), it only exist a reported study that found a high serum concentration of this pro-inflammatory cytokine in FM patients [83]. Other researchers found no differences in either serum [97], or in the cerebrospinal fluid [106]. However, it has also been reported in some studies that there is less IL-1β concentration in serum of people with FM [104,106,107]. No differences were observed in IL-1β concentration of the muscle in FM patients compared to healthy people control group [109].

IL-6 is one of the most important pro-inflammatory cytokines as induction mediators of acute phase proteins and earliest release by hepatocytes during inflammatory stimulus. According to Wallace et al. [10] IL-6 may be associated with hyperalgesia, depression, stress, fatigue and sympathetic nervous system activation. However, most published studies on the role of IL-6, show no differences in plasma concentration of IL-6 from FM patients compared to healthy women [10,95,97,99,100,102,106,107,110-112]. However, recent studies have reported high levels of IL-6 in FM patients [98,103,104]. While there are no differences in the concentrations of IL-6 either on the skin [108] or on the muscle [109] of FM patients compared to healthy people.

IL-8, a pro-inflammatory cytokine and chemokine (CXCL8), is a potent chemoatractant of neutrophils [94] (essential cells for the development of the inflammatory response, and is also a potent promoter of sympathetic pain [53,94]. The IL-8 has been found to be elevated in both serum and plasma of patients with FM [10,53,91,97-99,100,106,107,111,113]. However, while most studies which studied the role of IL-8 in fibromyalgia, found it high, there are also authors who have not indicated variations of this inflammatory cytokine in the blood of patients with FM [102,105,110,112,114,115]. However, unlike the variability of results found in the blood of FM patients regarding IL-8 concentration, in the cerebrospinal fluid of these patients, it has only been found high concentrations of IL-8, a cytokine which promoving and mediating the pain [106,107]. As with other pro-inflammatory cytokines, we have not found reported differences in the concentration of IL-8 either on the muscle or on the skin of patients with FM [108,109]. IL-18, inflammatory cytokine closely related to the inflammasome function, was found elevated in the serum of patients with FM [83].

Only one study has examined the levels of IL-17 in patients with fibromyalgia, finding that this cytokine is increased in FM patients [116].

The cytokine IL-10 is an anti-inflammatory cytokine that has been found increased both in serum [96,99,101,102] as in the cerebrospinal fluid [107] of FM patients, but other authors found no differences in the concentration of this anti-inflammatory cytokine between FM patients and the control group [10,97,98,100,112,114,117]. Even Menzies et al. [4] found that patients with FM had lower concentrations of IL-10 compared with healthy people. The same occurs with IL-8, IL-6 and TNF-α, IL-10 neither shows differences in the skin concentration present on patients with FM [108].

Respecting to another anti-inflammatory interleukin such as IL-4, the results are again contradictory since it has been reported increases on this cytokine concentration in serum [96] and in the cerebrospinal fluid [107] of patients with FM, other studies failed to establish differences between the study groups

[10,97,98,100,114,115,117]. Even Stürgill et al. [118] detected a decrease in the concentration of IL-4 in the serum of patients with fibromyalgia compared to the healthy group.

We only found two studies that evaluated the concentration of IL-1ra (anti-inflammatory cytokine) in patients with FM, being found, on both studies, increased serum concentration of this cytokine compared to the group of healthy people [10,107]. IL-5 has also been evaluated in the serum on patients with FM, being diminished on the study done by Kadetoff et al. [106] and Stürgill et al. [118], however, Behm et al. [112] found no differences in serum concentrations of this cytokine present in FM patients and healthy individuals. Stürgill et al. [118] also found a IL-13 concentration decrease in the blood of patients with FM. Similarly, Tsilioni et al. [103], in a very recent study have also found that IL-31 and IL-33 have lower serum concentrations in patients with FM than in healthy people. Finally, indicate that and regarding to IFN-γ, it was found a non-significant increase in the concentration of this inflammatory cytokine in serum of people with fibromyalgia compared to healthy control group [97].

Chemokines in FM

The chemoattractant cytokines or chemokines are a family of small soluble signaling molecules of about 70 amino acid residues with a molecular weight of 7-12 kDa. playing a crucial role on both homeostasis and disease. As disease modulators, chemokines play a role in a wide variety of inflammatory and immune responses through the chemoattraction of cells from the innate and adaptive immune response. [94,119]. They selectively control, often with specificity, events such as adhesion, chemotaxis and the activation of many types of leukocyte populations and subpopulations, therefore are important regulators of leukocyte traffic. Chemokines are functionally classified into three families: pro-inflammatory, homeostatic and with a mixed function [120]. Chemokines proinflammatory are regulated under inflammatory conditions and are involved in leukocyte recruitment to inflamed sites. Homeostatic chemokines are expressed constitutively in non-inflamed sites and are involved in the homeostatic migration and homing of cells under physiological conditions such as lymphocyte homing. Some chemokines have both properties, therefore are called mixed functions chemokines [121,122]. In addition, chemokines appear to be molecules with a role in the coordination of nociceptive events associated with the injury. Increase sensitivity to pain by direct action on chemokine receptors expressed in nociceptive neurons [123], and also serve to regulate the inflammatory response simultaneously acting on components of the nervous system [124].

The fact that the main symptom of fibromyalgia is chronic widespread pain, suggests that chemokines, such as inflammatory mediators and nociceptor's modulators [123], may be involved in the pathophysiology of this disease. In fact, in pain mechanisms, chemokines increase sensitivity directly acting on its receptors (which are present along the entire path of pain - in peripheral nerves,the site of damage to nerves, dorsal root ganglia and spinal cord) [123]. So, Zhang et al. [114] reported high plasma levels of MCP-1 and eotaxin in patients with FM, while Garcia et al. [94] found high serum levels of proinflammatory chemokines (MIG, MDC, I-TAC, TARC and eotaxin), while evaluated homeostatic cytokines (PARC and HCC-4) had no variations between fibromyalgia patients and healthy people. However, other researchers have found decreases in MCP-1 serum concentrations of MCP-1 from patients with FM [105], and fractalkine [125], as well as decreases in the concentration of MCP-1 on skin biopsies from FM patients [126]. While Behm et al.

[112] found no differences in serum levels of MCP-1, MIP-1α, MIP-1β of FM patients and healthy people.

Pentraxins in FM

Inflammatory mediators such as IL-6, also trigger non-specific acute phase response which on the synthesis of a series of plasma proteins (about 40) rapidly increases in response to released inflammatory mediators [23,127,128].

The C-reactive protein (CRP) and Pentraxin 3 (PTX3) are two of these acute phase proteins, and the more characteristic in humans. Both proteins are pentraxins, then PTX3 is a long chain pentraxin and is produced by innate immunity cells and vascular cells in response to inflammatory signals. While CRP (prototype of short chain pentraxin) is produced in a systemic level by hepatocytes, PTX3 is produced by a wide range of different cell types and performs its functions locally. Recent studies suggest that PTX3 may be a new marker of innate immunity and inflammation, which quickly reflects the tissue and vascular inflammation [129]. While CRP is a cardiac marker, linked to the damage and necrosis of cardiac muscle tissue and also linked to the tissue repair [129]. Therefore, regarding to the role that CRP plays in fibromyalgia, it has been found elevated serum levels in FM patients compared to healthy levels in almost all studies [4,97,130-138]. Only on three studies, published until now, it was not reported differences on the CRP´s concentration on FM patients and healthy individuals [139-141].

Regarding long chain pentraxins, like PTX3, only two studies have been published, the first by Skare et al. [142] which indicates that FM patients have high concentration of PTX3 comparing with healthy people, a fact supported by the results shown by Garcia et al. [143] demonstrating that isolated monocytes from FM patients produce more quantity of PTX3 than monocytes of healthy individuals.

Lipid Mediators

Lipid mediators (eicosanoids and platelet-activating factors) are derived from phospholipids, such as phosphatidylcholine, that are present in the inner leaflet of cellular membrane. After activation by intracellular Ca^{2+} ions, cytosolic phospholipase A2 generates arachidonic acid an lysophosphatidic acid. Arachidonic acid is metabolized to form eicosanoids either by cyclooxygenase (COX-1 and COX-2), which generate prostaglandins and tromboxanes, or by lipoxygenases, wich generate leukotrienes and lipoxins [23,79].

The PGE2 and PGI2, in turn cause vasodilation, and PGE2 is also hyperalgesic and a potent inducer of fever [144]. Until now, few studies have been made regarding the role of eicosanoids in the pathophysiology of FM, however and on two studies, PGE2 a hyperalgesic prostaglandin was been reported elevated in the blood of FM patients [145,146], while Sambrorsky et al. [147] found lower prostaglandin concentrations in the blood of patients with FM. However, Topal et al. [148] reported elevated serum levels of PGF2α in FM patients when compared with concentrations achieved on healthy people. Ardic et al. [145] found elevated levels of LTB4 in the plasma of people with FM, results which are according with those found by Hedenberg-Magnuson et al. [146] who found a higher expression of this leukotriene in the muscle of patients with FM.

Concerning to platelet-activating factors, which are generated by a acetylation of lysophosphatidic acid and activate several process that

ocurr during the inflammatory response, including recruitment of leukocytes, vasodilation and vasocontriction, increased vascular permeability and platelet activation [23,79,80], we only found a recent study by Fais et al. [52] who reported a decrease in the blood concentration of PAF-AH (platelet activating factor acetyl hydrolase) in patients with FM regarding the control group, accompanied by a high concentration of sPLA2 (secretory phospholipase A2).

Inflammatory Effectors Altered in Fibromyalgia

Once reviewed the altered inflammatory mediators in FM, and since most part of them are altered in the blood of patients with FM, it is logical to think that these inflammatory mediators may be altering and acting on the functional states of blood phagocytes (monocytes and neutrophils), to promote the elimination of inflammatory inductors, and adapt to the new physiological state and try to restore both cellular homeostasis and tissue homeostasis of the organism.

Thus, monocytes play a critical role on tissue homeostasis, protective immunity, and both promotion and resolution of the inflammation. They and their descendants, macrophages and dendritic cells are essential for innate and adaptive immune response to pathogens. Monocytes exert many of their functions outside the vascular compartment; therefore, it is crucial both traffic and migration of these cells. The recruitment of monocytes from the blood to the site of injury or infection and its diapedesis across the endothelium (also called extravasation) are crucial events on early inflammation, followed by monocyte differentiation and subsequent events of the inflammatory response [149].

Moreover, neutrophils, the most abundant human immune cells, are rapidly recruited to inflammation sites. Neutrophils are fast and powerful phagocytes. The neutrophil recruitment and activation are key events in acute inflammatory diseases. Neutrophil infiltration is not limited to infectious conditions and it also occurs on sterile environments, mediated by a migratory multistep sequence, involving the inflammasome and chemokines. Physiologically, acute neutrophilic inflammation is followed by a resolution phase, important for tissue homeostasis. If these resolution mechanisms fail, neutrophils lead to chronic inflammation, characterized by the release of proteases and oxidants that produces tissue injury [150]. So, functional alterations of these innate immune response and inflammatory cells described so far on fibromyalgia patients, are described below.

Altered Functionality of Neutrophils in Fibromyalgia

Neutrophils from FM patients have a greater ability to chemotaxis [54], phagocytosis [53,54] and microbicide capacity [54] than neutrophils from healthy women along with a higher level of oxidative stress [151], reflected in an increased peroxide hydrogen production (H2O2) [53]. Macedo et al. [152] reported that neutrophils from FM patients expressed greater amount of L-selectin (CD62L) than neutrophils of healthy people, however, Kauffmann et al. [153] indicated that these cells from fibromyalgia patients express less quantity of adhesion molecules, such as L-selectin the (CD62L) and β2-integrin (CD11b/CD18). However, these cells do not either exhibit altered production of inflammatory chemokines [154] or PTX3 [143]. What seems clear is that there is an alteration of the neutrophil functional status in the FM, probably due to the large number of inflammatory mediators in the blood of patients with FM. Thus, this neutrophil functional status altered and activated throw ROS production, may be feeding back the inflammatory circuit.

Altered Functionality of Monocytes in Fibromyalgia

Concerning to monocytes, they present a generalized alteration on production of the inflammatory mediators. So, monocytes, isolated by magnetic separation from the rest of peripheral blood mononuclear cells (PBMC), produce greater amounts of IL-1β [54,91,135], TNF-α [54,91,135], IL-6 [54,91,135], IL-18 [54,91] and IL-10 [54,91,135]; also, these cells from women with FM produce a greater amount of inflammatory chemokines, such as MCP-1 (CCL2) [54], eotaxin (CCL11), MDC (CCL22), GRO-α and (CXCL1) [154]. This increase of the chemokines, at the local level, produced by monocytes from FM patients would be stimulating the sensation of pain in these patients because chemokines increase pain sensitivity by directly acting on their receptors (wich are present along the entire pathway of pain in peripheral nerves, the sites of nerve damage, the dorsal root ganglia and the spinal cord) [123], as most of these chemokines are molecules that mediate both inflammatory and neuropathic pain [124,155,156]. Only one study has reported that monocytes from patients with FM produce less RANTES than monocytes of healthy individuals [54], while Garcia and Ortega [125] found no difference in the production of fractalkine (CX3CL1) by monocytes on FM patients and healthy people. However, recently it has been indicated that isolated monocytes from patients with fibromyalgia [143] produce more quantity of an acute phase protein, pentraxin 3 (local inflammation marker) than monocytes of healthy people. In studies with PBMC (peripheral blood mononuclear cells), it has also been indicated increased production of IL-6 [10] and IL-1β [83] by PBMC of patients with FM. However, other studies have found no differences in the production of TNF-α, IL-1β and IL-10 by PBMCs from patients with FM and healthy people [10,156]. It has also been suggested that PBMC of patients with fibromyalgia have lower intracellular ATP concentrations than PBMC of healthy individuals, which indicates an energy dysfunction and metabolic stress in these cells. In summary, monocytes from women with FM show an alteration in the release of inflammatory chemokines and cytokines. The altered chemokines may be mediating both the stimulation of the neutrophil phagocytic process and pain in FM patients [94]. Thus, non- lethal malfunctions of the innate immunity could be ocurring in FM wich would provoke low-grade inflammation, where monocytes may play a prominent role [94].

Conclusion

Since proper functionality of monocytes and neutrophils is essential to carry out a good innate immune response and therefore develop a good overall immune response, in view of these precedents, it appears that in fibromyalgia we can find dysregulated cellular bases of innate surveillance. Therefore, it could have been producing in women with FM, an alteration in the cellular and tissue homeostasis, affecting the immune system cells, leading to a graduated para-inflammation situation. Sometimes, closer to the homeostatic state (less presence of pain and associated symptoms) and other times closer to the classic inflammatory state (intense pain situations).

This para-inflammatory state in the FM, could be due to a (non-lethal) permanent deregulation of innate immune system, caused for situations of high chronic stress (as some studies on risk factors of FM seem to suggest), that will lead to some cells to a strong state of cellular stress, triggering cell death by pyroptosis, releasing inflammatory inductors and/or DAMPs that activate the inflammasome, it induces the release of a plethora of inflammatory mediators, that produce and regulate inflammatory pain, which finally alter the cellular functionality of phagocytes, which are unable to resolve inflammation

and feeding back through produced mediators the inflammasome activation.

In future studies it would be interesting to know whether this innate immune cellular dysregulation is the cause of the possible para-inflammation underlying on fibromyalgia, or the effect of a chronic low-grade inflammation due to other causes and triggering a functional alteration of phagocytes.

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