Cytokines and Pain

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Summary: de Oliveira CMB, Sakata RK, Issy AM, Gerola LR, Salomão R – Cytokines and Pain.

Background and objectives: Cytokines are necessary for the inflammatory response, favoring proper wound healing. However, exaggerated proinflammatory cytokine production can manifest systemically as hemodynamic instability or metabolic derangements. The objective of this review was to describe the effects of cytokines in pain.

Contents: This article reviews the effects of cytokines in pain. In diseases with acute or chronic inflammation, cytokines can be recognized by neurons and used to trigger several cell reactions that influence the activity, proliferation, and survival of immune cells, as well as the production and activity of other cytokines. Cytokines can be proinflammatory and anti-inflammatory. Proinflammatory cytokines are related with the pathophysiology of pain syndromes. Cells that secrete proinflammatory (IL-1, IL-2, IL-6, IL-7, and TNF) and anti-inflammatory (IL-4, IL-10, IL-13, and TGFβ) cytokines, the functions of each cytokine, and the action of those compounds on pain processing, have been described.

Conclusions: Cytokines have an important role in pain through different mechanisms in several sites of pain transmission pathways.

Keywords: Cytokines; Interleukins; Nociceptors; Pain.

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INTRODUCTION

Cytokines are hydrosoluble extracellular polypeptides or glycoproteins ranging from 8 to 30 kDa. They are produced by several types of cells, at the site of injury, and immune cells, through mitogen-activated protein kinases. Unlike classical hormones, cytokines are not stored as preformed molecules, acting especially by paracrine (in neighboring cells) and autocrine (in the producing cells) mechanisms [1,2]. Different types of cells secrete the same cytokine, and a single cytokine can affect several types of cells, which is called pleiotropy. Cytokines are redundant in their activities, i.e., similar actions can be triggered by different cytokines. They are frequently formed in cascades, i.e., one cytokine stimulates its target-cells to produce more cytokines [3]. Those substances bind specific receptors, activating intracellular messengers that regulate gene transcription. Therefore, cytokines influence the activity, differentiation, proliferation, and survival of immune cells, as well as regulate the production and activity of other cytokines that can increase (proinflammatory) or decrease (anti-inflammatory) the inflammatory response. Some cytokines can have a pro- (Th1) or anti-inflammatory (Th2) actions, according to the microenvironment in which they are located. Among proinflammatory cytokines, we can mention interleukins (IL) 1, 2, 6, 7, and TNF (tumor necrosis factor). Anti-inflammatory cytokines include IL-4, IL-10, IL-13, and TGFβ (transforming growth factor) [2,4].

Cytokines are mediators necessary to conduct the inflammatory response in sites of infection and injury favoring proper wound healing. However, exaggerated production of proinflammatory cytokines at the site of injury can manifest systemically as hemodynamic instability or metabolic derangements. After severe injury or infection, exacerbated and persistent Th1 cytokine response can contribute for target-organ damage leading to multiple organ failure and death. Th2 cytokines can minimize some of those undesirable effects [1,2,4].

Since it is not possible to classify cytokines according to the cell of origin or to their biologic function, they were grouped in interleukins (IL, numbered sequentially from IL-1 to IL-35), tumor necrosis factors (TNF), chemokines (chemotactic cytokines), interferons (IFN), and mesenchymal growth factors [2,5].

INTERLEUKIN-1 (IL-1)

Interleukin-1 is produced primarily by macrophages and monocytes, as well as by non-immune cells such as activated fibroblasts and endothelial cells, during cellular damage, infection, invasion, and inflammation. There are two known types: IL-1α and IL-1β, each with 31 to 33 kDa. They act on the same receptors, IL-1RI and IL-1RII. IL-1RI is considered the active receptor, while IL-1RII does not have a transduction molecule being functionally inactive. IL-1α is markedly associated to cell membranes and it exerts its action through cell contact. IL- 1β is synthesized as a precursor protein (Pro-IL-1 β), which is not secreted in its active form until it is metabolized by the enzyme caspase-1. Recently, it was discovered that

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IL-1β is expressed in nociceptive neurons of the dorsal root ganglia [1,3,5].

IL-1β produces systemic inflammation through activation of cyclooxygenase-2, with formation of PGE_2 in the anterior hypothalamus causing fever. It also produces substance P (SP), nitric oxide (activating the enzyme oxide nitric synthase), and endothelial adhesion molecules. It is important in the development and maintenance of postoperative pain [3,5,6].

IL-1RA (receptor antagonist) is also released during tissue damage and it does not have agonist action both *in vitro* and *in vivo*. Thus, it competes with the same receptors of IL-1, behaving as an endogenous auto-regulator [4].

Although it has a plasma half-life of only 6 minutes, recently it has been suggested that IL-1 is important in the development and maintenance of postoperative pain [1,6].

INTERLEUKIN-2 (IL-2)

Interleukin-2 is a 15 kDa protein produced mainly by T CD4 cells and, in lesser amounts, by T CD8+ cells. It exerts its actions through IL-2Rα, IL-2Rβ, and IL-2Rγ receptors using the intracellular JAK/STAT (Janus family of tyrosine kinases/transcription factors) pathway to stimulate the growth and proliferation of T lymphocytes and B cells. It also induces the production of other cytokines, such as IFNγ and TNFβ resulting in the activation of monocytes, neutrophils, and natural killer cells. Thus, it is evident that IL-2 contributes for the generation and propagation of antigen-specific immunologic responses [4,5]. Since its plasma half-life is less than 10 minutes, IL-2 is usually not detected in acute injuries [1].

Although *in vitro* studies indicate that IL-2 is proinflammatory, its intraplantar injection has an anti-hyperalgesic effect [7]. The administration of IL-2 in the locus ceruleus of rats inhibited the noxious sensation [8].

IL-2 has been widely used in clinical applications, such as oncologic therapy, immunodeficiency, and graft rejection [9-12].

INTERLEUKIN-4 (IL-4)

Inteleukin-4 is a 15 kDa glycoprotein with anti-inflammatory properties that is produced by CD4 T lymphocytes, mastocytes, eosinophils, and basophils. It exerts its action on T and B lymphocytes, natural killer cells, mastocytes, synoviocytes, and endothelial cells using the JAK/STAT pathway. It induces B lymphocyte differentiation to produce IgG and IgE, important immunoglobulins in allergic and anti-helminth response. It affects activated macrophages reducing the effects of IL-1, TNFα, IL-6, and IL-8 and inhibiting the production of oxygen free radicals. Besides, it increases macrophage susceptibility to the effects of glucocorticoids [2,4].

Interleukin-4 has therapeutic potential in many clinical situations, such as psoriasis, osteoarthritis, lymphoma, and asthma [13-16].

INTERLEUKIN-6 (IL-6)

Interleukin-6 is a 22- to 27-kDa glycoprotein secreted by many types of cell, such as macrophages, monocytes, eosinophils, hepatocytes, and glial cells, and TNFα and IL-1 are potent inducers. It causes fever and it activates the hypothalamic-pituitary-adrenal axis using α receptors (IL-6R α) and the gp130 subunit (glycoprotein 130, member of the class I cytokine receptor superfamily). It has a structural relationship with IL-4, leukemia inhibitory factor, erythropoietin, and ciliary neurotrophic factor [2,4].

This interleukin is one of the earliest and important mediators of induction and control of acute phase protein synthesis and release by hepatocytes during pain stimuli, such as trauma, infection, surgery, and burns. After an injury, plasma concentrations of IL-6 are detectable within 60 minutes, with a peak between 4 and 6 hours, and it can persist for up to 10 days. It is considered the most relevant marker of the degree of tissue damage during a surgical procedure in which excessive and prolonged increase is associated with greater postoperative morbidity [1,17-19].

Interleukin-6 is a proinflammatory cytokine that promotes neutrophil maturation and activation, macrophage maturation, and differentiation/maintenance of cytotoxic T-lymphocytes and natural killer cells. Besides, it activates astrocytes and microglia, and it regulates the expression of neuropeptides after neuronal damage, contributing for their regeneration. However, it also has anti-inflammatory properties, since it releases soluble receptors of TNF (sTNFRs) and IL-1AR [1,4,5].

INTERLEUKIN-10 (IL-10)

Interleukin-10 is an 18-kDa non-glycosilated peptide synthesized in immune cells and neuroendocrine and neural tissues. Similar to interferon receptors, its receptor (IL-10R) belongs to the class II cytokine receptor family. Production of IL-10 is hindered by several cytokines, such as IL-4, IL-13, and IFNγ, and also by its own autoregulation [1,2,5].

It inhibits proinflammatory cytokines, especially TNF, IL-1, and IL-6, produced by activated macrophages and monocytes, stimulating endogenous production of anti-inflammatory cytokines. Besides, it increases the proliferation of mastocytes and prevents the production of IFNγ by natural killer cells [3,4].

Its suppressive effects on Th1 cells can be clinically useful in the prevention of graft rejection and to treat T-cell-mediated autoimmune disorders, such as multiple sclerosis and type-1 diabetes mellitus. A beneficial effect can also be observed in sepsis, rheumatoid arthritis, and psoriasis. On the other hand, IL-10 antagonism can show satisfactory effect during activation of polyclonal B-cells and hyperglobulinemia in patients with AIDS (acquired immunodeficiency syndrome) [20-24].

INTERLEUKIN-13 (IL-13)

Interleukin-13 has similar structural and functional characteristics to IL-4, from which it differs since it does not stimulate

the proliferation of mitogen-induced blasts or T-lymphocyte clones and it does not promote the expression of CD8α in CD4 T lymphocyte clones. It is an anti-inflammatory cytokine produced mainly by CD4 T-cells. It affects B-lymphocytes and monocytes, inhibiting the production of nitric oxide and several cytokines, such as IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12, macrophage inflammatory protein-1α, IFNα, and TNFα. Besides, it increases the synthesis of IL-1AR [1,4].

INTERLEUKIN-17 (IL-17)

Currently called IL-17A, it is the prototype of the IL-17 family. It is a homodimeric protein with 155 amino acids bound to a disulfide radical. It is produced predominantly by CD4 T-lymphocytes, acting as a 35-kDa homodimer in T lymphocytes. Interleukin-17A is proinflammatory, leading to the formation of IL-6 and IL-8 (chemokine) and intercellular adhesion molecule in human fibroblasts [2,4].

TUMOR NECROSIS FACTOR ALPHA (TNFα)

Tumor necrosis factor α, also known as cachectin, is a proinflammatory cytokine produced mainly by monocytes, macrophages, and T-lymphocytes that are abundant in the peritoneum and splanchnic tissue. It is also present in neurons and glial cells, with functions important both in inflammatory and neuropathic hyperalgesia. Tumor necrosis factor exists in two forms: a 26-kDa transmembrane and a secretory with 17 kDa, and both are biologically active. It is structurally related to lymphotoxin-α (LI α, also known as TNFβ), which has the same receptors, TNFR1 (55 kDa) and TNFR2 (75 kDa). TNFR1 is expressed exclusively on neurons and it is associated to the majority of the biological effects of TNFα, including inflammatory responses and apoptosis. TNFR2 is manifested mainly in macrophages and monocytes of the dorsal root ganglia, stimulating proliferation of T-lymphocytes, fibroblasts, and natural killer cells [1-3].

After a surgical procedure, trauma, or during infections TNFα is one of the earliest and potent mediators of the inflammatory response. Although it has a half-life of only 20 minutes, it is enough to cause important metabolic and hemodynamic changes and activate other cytokines distally. Tumor necrosis factor α is a potent inducer of muscular metabolism and cachexia by stimulating lipolysis and inhibiting lipoprotein lipase. Other TNFα actions include activating coagulation, stimulating the expression or release of adhesion molecules, PGE_2, platelet activating factor, glucocorticoids and eicosanoids, and influencing apoptosis [4,5].

Tumor necrosis factor α has great affinity for TNF soluble receptors (sTNFRs), derived from the extracellular domains of TNFRs. Activation of sTNFRs produces endogenous antagonist response activity, which antagonizes excessive systemic TNFα activity. However, note that sTNFRs can cause undesirable effects, since they behave as transporters or bioactive reserves of TNFα in the circulation [1].

TRANSFORMING GROWTH FACTOR β (TGFβ)

Transforming growth factor β is an anti-inflammatory cytokine with approximately 13 kDa and composed of 112 amino acids. It has five different isoforms: TGFβ1 to β5. Transforming growth factor β1 inhibits production of IL-1, IL-2, IL-6, and TNF, and it induces IL-1AR. Its messenger RNA is induced after axotomy and it may be involved in a negative retro-feeding mechanism to limit glial activation. Transforming growth factor β1 also prevents macrophages from synthesizing nitric oxide, which is strongly implicated in the development of neuropathic pain [3,4].

CYTOKINES AND NOCICEPTION

Pain and the immune system influence each other, making it difficult to determine whether blocking nociception contributes for a reduction in the production of proinflammatory cytokines or vice-versa, with the reduction in the formation of proinflammatory cytokines resulting in less severe pain [25].

The traditional idea of post-trauma microenvironment reveals that leukocyte migration associated to inflammation is responsible for secreting chemical mediators that produce pain. However, current evidence suggests that the function of the inflammatory response in pain generation is not limited only to the effects produced by the migration of leukocytes. Thus, it is believed that proinflammatory cytokines that participate in the noxious process may originate from immune, neuronal, and glial cells (microglia and astrocytes), both in the peripheral and central nervous system, and that those molecules can trigger short- and long-term effects, with occasional chronic hyperexcitability and changes in phenotypic expression of nociceptors, abnormal processing of noxious signals, and exacerbation of pain processes. Those effects are caused directly by cytokines or mediators formed under their control [26-28].

Interleukin-1β and TNFα, the first cytokines to be formed after tissue damage or infection, affect directly specific receptors on sensorial neurons, leading to the "cascade" synthesis of other effectors, such as other cytokines, chemokines, prostanoids, neurotrophins, nitric oxide, kinins, lipids, adenosine triphosphate (ATP), and members of the complement pathway. On their turn, those elements cause glial cell proliferation and hypertrophy in the central nervous system, releasing of relevant proinflammatory cytokines, TNFα, IL-1β, and IL-6, forming a complex network of independent activation [3,25,27,28].

Tumor necrosis factor α reduces the activation threshold of type C peripheral nerve fibers for mechanical stimuli through extravasation of plasma, generating mechanical allodynia. It increases the ion currents in tetrodotoxin-resistant sodium channels in neurons of the dorsal root ganglia (DRG) by activating TNFR1 receptors and p38 mitogen-activated protein kinase (p38 MAPK). This, in general, can be found by $Na_v1.8$ sodium channels in DRG and its direct phosphorylation causes an increase in current density, contributing to generate inflammatory and neuropathic pain. Tumor necrosis factor also affects the conductance of potassium channels by ac-

tivating PKC, which affects the capacity of glial cells to allow the outflow of intracellular potassium and remove glutamate released after a stimulus, resulting in greater neuronal vulnerability [2,29].

Substance P behaves as a neurotransmitter, neuromodulator, or trophic factor by binding to neurokinin-1 receptors. Calcitonin-gene related peptide (CGRP) is a potent vasodilator and it is also involved in pain induction. Interleukin-1β stimulates the release of SP and CGRP, while IL-6 favors the synthesis of SP in sensorial neurons. Tumor necrosis factor induces SP in sympathetic ganglia. Interleukin-1β also activates B1 bradikinin receptors, generating thermal hyperalgesia. Tumor necrosis factor and IL-1β activate B2 receptors, causing inflammatory hyperalgesia. Bradikinin itself can induce the secretion of TNF and IL-1β from macrophages, forming a vicious nociception cycle. Note that isolated IL-1β is incapable of stimulating DRG neurons; however, along with IL-6 and TNFα, it produces a rapid increase in sensitivity of TRPV1 and release of CGRP, leading to thermal sensitization. Tumor necrosis factor, IL-1β, and IL-6 are potent cyclooxigenase-1 inducers and consequently of PGE_2 both at the site of damage and in the spinal cord increasing neuronal sensitivity to chemical, thermal, and mechanical pain stimuli. Besides, several actions of TNFα and IL-1β are performed by the binding of NGF (nerve growth factor) and tyrosine kinase-A receptors (trkA). In inflamed tissues, NGF promotes macrophage proliferation, degranulation, and release of inflammatory mediators including NGF itself generating a self-activating cycle. Nerve growth factor has both peripheral and central action in the nervous system by genetic alteration and post-translational receptor and ion channels (such as TRPV1, PKA, PKC, MAPK, and tetrodotoxin-resistant sodium channels) regulation, inducing thermal and mechanical hyperalgesia. Nerve growth factor can also cause peripheral sensitization through the activation of 5-lypoxigenase, which converts arachidonic acid in leukotrienes that cause nociceptive afferents to become excitable to thermal and mechanical stimuli [2,25,28,30-32].

Chemokines are small proteins secreted by peripheral blood cells, neurons, or glial cells, exerting most of their function through the activation of G protein-coupled receptors (CCR1, CCR2, CCR5, CXCR3, CXCR4, and CX3CR1). They are responsible primarily by migration of leukocytes to the site of tissue damage or infection, but they also participate in synaptic transmission and in the formation of second messenger systems in neurons and glial cells, favoring the noxious process. Based on the presence and position of the first cysteine residues, they are classified in four groups: CC chemokines (RANTES, MCP-1/CCL2, MIP-1α, and MIP-1β), which have two adjacent cysteine residues; CXC chemokines (IL-8 and SDF-1), with one amino acid between two cysteine residues; C chemokines (lymphotactin); and CX3C chemokines (fractalkine), with three amino acids between two cysteines [3,4,33].

CXC chemokines, such as SDF-1, exert their actions through CXCR4 receptors in neurons and/or astrocytes, influencing the release of glutamate, affecting neuronal excitability and apoptosis. Interleukin-8 causes GABA expression in central synapses. MCP-1/CCL2 modifies negatively currents induced by GABA and/or facilitates excitotoxic events in rat central nervous system [2,34].

MCP-1/CCL2 is distributed mainly in neurons of the dorsal root ganglia and dorsal horn of the spinal cord. It has high affinity for CCR2 receptors and it is a potent chemotactic and monocytes, T-cells, natural killer cells, and eosinophils activator. In the dorsal root ganglia, it can stimulate primary nociceptive neurons by an autocrine and/or paracrine process, perhaps due to a crossed intraganglion excitation phenomenon. Besides, MCP-1/CCL2 synthesized in the dorsal root ganglia is dislocated for the dorsal horn of the spinal cord, where it changes the activity of post-synaptic neurons and glial cells, facilitating noxious transmission [2,3].

The chemotactic actions of RANTES affect several types of leukocytes, including monocytes, macrophages, microglia, T-cells, eosinophils, basophils, and neurons of the dorsal root ganglia through CCR1, CCR3, and CCR5 receptors. RANTES is important in painful peripheral neuropathies associated with HIV-1, increasing the inflow of calcium in sensorial neurons through CCR5 [35].

MIP-1α has greater affinity for CCR1, CCR3, and CCR5 receptors, producing calcium mobilization in astrocytes, neurons, and leukocytes, increasing excitability. Particularly, the activation of CCR1 receptors causes desensitization of dorsal root ganglia neurons to μ opioid receptor agonists, most likely by reducing the amount of those receptors on the membrane. It also affects TRPV1 receptors of nociceptive neurons, exacerbating thermal sensitivity [2,28].

Fractalkine is the only member of the CX3C family, composed by 373 amino acids. It is expressed on the plasma membrane of endothelial cells, macrophages, dendritic cells, and almost all sensorial neurons and neurons of the dorsal horn of the spinal cord. After the action of cathepsin S, its soluble form is released and behaves as a chemotactic agent for T-cells, monocytes, natural killer cells, and microglia. It is assumed that soluble fractalkine activates CX3CR1 receptors, present exclusively on microglia of the central nervous system, leading to phosphorylation of the enzyme p38 MAPK, with the consequent release of inflammatory mediators, establishing a positive retro-feeding system that can contribute for a state of chronic pain [2,33].

In the spinal cord, TNF and IL-1β cause an increase in the activity of AMPA or NMDA receptors, while IL-1β and IL-6 inhibit GABA- and glycine-induced ion currents in Rexed lamina II nociceptors, demonstrating clearly that those proinflammatory cytokines favor the increase in neuronal excitability [36]. Tumor necrosis factor also reduces the expression of the glutamate transporter gene and reuptake of glutamate by other glial transporters, stimulating spinal processing of noxious stimuli [37]. In hippocampal neurons, TNF promotes greater expression of the GluR1 subunit of AMPA receptors on cell surface. This is accompanied by a reduction of GluR2 subunit that supposedly is the result of fast appearing calcium permeable AMPA/KA channels and lower concentration of impermeable calcium AMPA receptors on neuronal

membrane. The increase in expression of AMPA receptors is mediated by TNFR1 and requires the action of inositol triphosphate kinase. Those changes on the density of AMPA receptors induced by glial TNF could be responsible for the rearrangement of neuronal synapses [2].

Unlike the nociceptive effects on proinflammatory cytokines described, TNFα, IL-1β, and IL-6 also stimulate the synthesis of opioids receptors and peptides in dorsal root ganglia, that are axonally transported to inflamed peripheral tissues, contributing for analgesia. With the same intent, chemokines increase the number of opioid-carrying leukocytes in the site of injury [38,39].

CONCLUSION

Several clinical studies have used antibodies to neutralize specific cytokines in the treatment of strokes, Alzheimer's disease, autoimmune disorders, wound healing, and amyotrophic lateral sclerosis, as well as the use of local or systemic anti-inflammatory cytokines or proinflammatory cytokine antagonists (such as glucocorticoids, thalidomide, and pentoxiphylline) in chronic pain. Those antagonists or anti-inflammatory cytokines could break the hyperexcitability cycle of sensorial neurons, promoting a new non-opioid therapeutic approach for pathologic pain caused by inflammation or peripheral nerve damage [2,3,5,28].