Send Orders for Reprints to reprints@benthamscience.ae

Current Rheumatology Reviews, 2015, *11*, 109-115

Cytokine and Immune System Abnormalities in Fibromyalgia and Other Central Sensitivity Syndromes

Roland Staud*

Department of Medicine, Gainesville, Florida 32610, USA

Roland Staud

Abstract: The nervous system as well as the immune system use common signaling molecules for intra- and inter-system communications. Specifically, both entities produce a similar array of peptide and non-peptide transmitters that act on a common set of receptors present in the two systems. One important set of such signaling molecules are cytokines. The wide distribution of cytokine receptors throughout the body, including the immune and the nervous system allows direct communication between these two entities. In addition to cytokines the nervous system and immune system also communicate with each other using shared ligands such as neurotransmitters and neuroendocrine hormones, and their respective receptors. Some of the most important clinical interactions between these two systems are associated with the "sickness response" as well as pain and analgesia. This "sickness response" which has been frequently attributed to inflammatory cytokines, strongly resembles the core symptoms of fibromyalgia and other Central Sensitivity Syndromes (CSS). Therefore a large number of research studies have focused on the relationship between peripheral cytokines and CSS. However, a lack of consistent associations was observed between CSS symptoms and peripheral cytokines which seem to suggest that maybe cytokines abnormalities of the central nervous system contribute to the pathogenesis of these illnesses. Better knowledge of cytokine –nervous system interactions may ultimately benefit the development of interventions that improve CSS manifestations including the "sickness response" and chronic pain.

Keywords: Central sensitivity syndromes, central sensitivity, clinical pain, cytokines, inflammation.

INTRODUCTION

Cytokine is a term used to describe a broad range of structurally diverse molecular families and individual proteins best known for their critical roles in immune system function. They are small proteins (5–20 kDa) that play an important role in cell signaling. They are important in health and disease, specifically in host responses to infection, trauma, sepsis, immunity, cancer, and reproduction. Based on their functional characteristics, these proteins have been subdivided into families, such as chemokines, interferons, interleukins, lymphokines, tumor necrosis factor, etc. They bind to specific receptors that affect the behavior of target cells. They include pleiotropic molecules with diverse and cell type specific activities. After binding to specific receptors cytokines regulate cell activation, hematopoiesis, apoptosis, cell migration, and cell proliferation. Although their main function is regulation of the immune system, specifically humoral and cell-based immune responses, they also affect the maturation, growth, and responsiveness of many cell populations. Cytokines production occurs in a range of cells, including immune cells (macrophages, B-lymphocytes, T-lymphocytes and mast cells), as well as endothelial cells, fibroblasts, and various stromal cells; and a given cytokine may be produced by more than one cell type [1]. The accurate detection of cytokines in peripheral tissues and blood requires sophisticated techniques and standardized protocols for sample preparation and storage have been developed to minimize the risk of sample instability [2-7]. Most investigators use multiplex immunoassays which employ flow cytometry and immunoassay methods thus allowing the measurement of multiple cytokines within a single sample [8].

Cytokines have not only been linked to immune responses but also to nociception and hyperalgesia [9, 10]. Much of the available evidence comes from animal models which demonstrated the pain modulatory function of pro- and anti-inflammatory cytokines [11]. In general, pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-6 appear to have mostly hyperalgesic effects, whereas anti-inflammatory cytokines like IL-4 and IL-10 seem to possess mostly analgesic properties.

Fibromyalgia (FM) and other central sensitivity syndrome (CSS) like irritable bowel syndrome (IBS), chronic fatigue syndrome (CFS), temporo-mandibular joint disorder (TMD) and migraine headache, are common chronic non-malignant pain disorders whose pathogenesis is only partially understood. Important bio-psycho-social factors contributing to these chronic pain syndromes include increased nervous system responsiveness to painful as well as non-painful stimuli, insomnia and distress [12-17]. Many prospective and retrospective studies have demonstrated sub-

*Address correspondence to this author at the Department of Medicine University of Florida College of Medicine, Gainesville, FL 32610-0221, USA; Tel.: (352) 273-9681; Fax: (352) 392-8483; E-mail: staudr@ufl.edu

stantial health-care costs associated with FM and other CSS [18, 19].

CYTOKINES AND PAIN

Many of the peripherally released cytokines are known to be pro-nociceptive and thus able to contribute to the development of pain [9]. Although there is currently no unifying hypothesis about the pathogenesis of FM and other CSS, several lines of evidence emphasize the relevant contributions of cytokines in the pathogenesis of chronic pain disorders. Besides being involved in virtually all aspects of both innate and adaptive immune responses, cytokines play an important role in diverse clinical symptoms such as fatigue, fever, sleep, stress, and pain. Leukocytes are a primary source of cytokines, but they are produced by many other cell types as well, including glia of the peripheral and central nervous system [20]. At least three types of glial cells appear to be involved in the development and maintenance of chronic pain syndromes. They include microglia and astrocytes of the central nervous system, and satellite glial cells of the dorsal root and trigeminal ganglia [21]. Glial signaling molecules, including cytokines have been shown to powerfully modulate excitatory and inhibitory synaptic transmission at presynaptic, postsynaptic, and extra-synaptic sites [22]. Cytokine production associated with acute or repetitive tissue injuries may be responsible for long-term activation of spinal cord glia and dorsal horn neurons, which may result in central sensitization.

Because cytokines are mostly involved in cell-cell interactions the significance of peripheral blood cytokine levels in patients with CSS and other chronic pain disorders is unclear. Compared to healthy controls, patients with peripheral neuropathies [9, 23], FM [24-26], CFS [27-29], or IBS [30] have been found to have increased peripheral blood levels of pro-inflammatory cytokines [31]. However, while some studies have reported good correlations between peripheral cytokine levels and chronic pain intensity [25, 32], others have not [24, 33]. Therefore several investigators studied cytokine concentrations in painful tissues, including muscles using microdialysis, an *in vivo* method for studying local tissue metabolites and signaling molecules. While several studies found significant cytokine elevations in painful muscles [34-36] others could not replicate these findings [37]. Overall, the results of these studies are controversial because of methodological differences which can strongly influence the measurements of cytokines levels. Thus better assay standardization will be necessary before controversies about the role of local tissue cytokines for CSS can be resolved [38].

CENTRAL SENSITIZATION AND PAIN

The term "central sensitivity syndromes (CSS)" has been proposed by Yunus for a group of overlapping illnesses without obvious structural tissue pathology including temporo-mandibular joint disorder (TMD), irritable bowel syndrome (IBS), chronic fatigue syndromes (CFS) chronic pelvic pain and fibromyalgia (FM) [39, 40]. The pathogenesis of these syndromes is only partially understood but involves long-term central sensitization (CS) of spinal and supraspinal neurons in the absence of apparent inflammation or neural lesions. CS most often manifests itself as pain hypersensitivity due to amplification of neural signaling within the central nervous system, more specifically as dynamic tactile allodynia, secondary punctate or pressure hyperalgesia, enhanced temporal summation, and pain aftersensations [41]. Such changes, however, are not specific for CSS but have also been reported in osteoarthritis, dental pain, neuropathic pain, and post-surgical pains [41]. CS is associated with enhanced excitability of dorsal horn neurons of the spinal cord and manifested by increased spontaneous neural activity, enlarged receptive fields, and augmented stimulus responses transmitted by large- and small-diameter primary afferent fibers [42] using glutamate and substance P as neurotransmitters [43]. Additionally, activation of N-methyl-D-aspartate (NMDA) receptors in the dorsal horn of the spinal cord is critical for CS, resulting in removal of a magnesium block from its Ca2+ channel, followed by influx of extracellular Ca2+ and phosphorylation of protein kinase C [44]. All these changes result in enhanced responsiveness of dorsal horn wide dynamic and nociception specific neurons which are crucial for the transmission of pain signals to higher order pain centers.

CYTOKINES IN FIBROMYALGIA SYNDROME

Although there is no evidence that FM is an inflammatory pain disorder, symptoms and signs associated with this syndrome have also been observed in inflammatory conditions. These symptoms include pain, allodynia, fatigue, hyperalgesia, insomnia, anxiety, and cognitive dysfunction, which also have been reported in infectious diseases and cancer patients [45]. Most of these symptoms have been attributed to cytokines which are thought to generate so-called "sickness behaviors" in FM and other CSS [46]. Sickness behaviors represent a set of behavioral and physiological changes that is considered as the adaptive response of the peripheral and central nervous system to stressful conditions involving the activation of the innate immune system [47]. Similarly, systemic injections of cytokines including IL-1, IL-6 and TNF-α have been shown to induce sickness behavior in animals [48]. Some studies of FM patients have suggested that at least a subgroup of FM patients may suffer from low-grade general [49, 50] or local neurogenic inflammation [51, 52] after increased levels of substance P (SP), calcitonin gene- related peptide (CGRP), and IL-8 were detected in the cerebrospinal fluid of FM patients [53-55].

Although for many years cytokines have been suspected to significantly contribute to FM, their precise role in the pathogenesis of this illness is still unknown. A connection between the pathogenesis of FM and cytokines was first suspected when abnormalities of hypothalamic-pituitary (HPA) axis function were first observed in FM patients [56], suggesting an important linkage between the HPA axis, sympathetic nervous system, and cytokines [57]. Neurotransmitters like acetylcholine are known to activate the HPA axis, whereas several cytokines including IL-1 and interferon alpha (IFN) can inhibit this neuro-endocrine axis [58]. Furthermore, increased levels of IL-1 have been associated with numerous FM-like symptom including fatigue, hyperalgesia, and low grade fever [59]. Several other cytokines, including TNF-α and IL-6 can trigger symptoms frequently observed

in FM patients like daytime sleepiness and pain [60]. The latter cytokine can also elicit cognitive dysfunction and worsen depression, two often disabling symptoms of FM [61, 62].

Several cytokines studies of FM patients used peripheral blood to examine the levels of inflammatory and anti-inflammatory cytokines like IL-1, IL-6, IL-8, TNF-α, and IL-10 [24, 25, 45]. A recent meta-analysis of 25 cytokine studies included 1,255 FM patients and 800 healthy controls [63]. Despite the large number of study subjects the general methodological quality of most studies was found to be low. Although FM subjects demonstrated higher serum levels of IL-1 ra, IL-6, and IL-8, compared to normal controls, the concentrations of most cytokines in peripheral blood samples were not different between patients and controls. Therefore more high quality studies will be necessary to adequately evaluate the pathogenetic role of cytokines for FM.

Other methods of cytokine measurements in the blood of FM patients include testing of cytokine gene expression patterns using quantitative real-time polymerase chain reaction [64]. When mRNA levels for IL-2, IL-4, IL-8, IL-10, TNF-α , and transforming growth factor (TGF) ß 1 were examined in peripheral blood of FM patients, the expression of anti-inflammatory cytokines IL-4 and IL-10 were found to be lower compared to controls [33]. However, the mRNA levels for IL-2, IL-8, TNF-α or TGF ß 1 were not different between groups. These findings suggested decreased anti-inflammatory and analgesic cytokine activity in FM. Furthermore, when peripheral blood mononuclear cells (PBMC) of FM patients were stimulated with lectins or phorbol esters [45], no differences in supernatant levels of IL-1 ß, IL-2, IL-10, serum IL-2 receptor, IFN- γ , and TNF-α were detectable between FM and controls. Only few cytokine levels, including IL-1ra and IL-6 were significantly higher after stimulating PBMC of FM patients compared to controls. However, these results could not be corroborated in a subsequent study of stimulated PBMC of CFS and FM patients using intracellular cytokine staining and flow cytometry [65]. In this study IL-1 a, IL-6, TNF-α, and IL-10 cytokine levels of PBMC were similar in patients and controls in either unstimulated or IFN-γ stimulated cells.

Other sources of cytokines and neuropeptides relevant for FM and other CSS may include activated microglia and astrocytes in the brain and spinal cord which are able to release IL-1, IL-8, nerve growth factor (NGF), and SP, all of which could perpetuate pain and "sickness-behaviors" of CSS patients [55, 57]. At this time, however, direct access to the central nervous system of CSS patients is difficult and mostly limited to testing of cerebrospinal fluid levels of cytokines.

CYTOKINE DYSREGULATION IN CHRONIC FATIGUE SYNDROME

The prevalence of chronic fatigue syndrome (CFS) is between 0.2% and 2.6% in the general population and similar to most other CSS, mostly women are affected [66-68]. Its main characteristic is severe and disabling fatigue for more than 6 months [69] as well as a number of other symptoms including musculoskeletal pain, sleep disturbance, impairment in short term memory and concentration, sore throat, and headaches [70]. Patients with CFS frequently report exacerbation of fatigue after any form of stress, including physical and/or mental exertion [71]. The long-term prognosis of CFS is poor as only few patients return to normal functioning over time [72].

The pathogenesis of CFS is only partially understood but is considered to have a significant immunological basis [73, 74]. Several investigators reported impaired functioning of natural killer (NK) cells in CFS patients [68, 75] with deficiencies of perforin [76], increased inflammation [77, 78], and elevated levels of pro-inflammatory cytokines [79]. Chronic infections, particularly of viral origin have been implicated, including infections with Epstein-Barr virus (EBV) and several herpes viruses [80, 81]. Similar to other CSS, many of the symptoms experienced by CFS patients strongly resemble the ''sickness behaviors" that can be induced by the administration of pro-inflammatory cytokines [82]. At least some of the CFS symptoms are thought to be the effects of pro-inflammatory cytokines on brain cellular targets. However, over the last 20 years a number of conflicting reports have been published on cytokine abnormalities in patients with CFS. Early reports of elevated inflammatory biomarkers including C-reactive protein (CRP), beta 2-microglobulin, neopterin [83], and TNF-α [84] seemed to implicate chronic inflammation as relevant for the CFS pathogenesis, but subsequently such findings could not be consistently replicated [85]. Although some of these discrepancies may be due to patient variables, most seemed to arise from methodological differences. In particular, cytokines have been evaluated by investigators using various methods including a) direct immunoassay of serum or plasma, b) quantitative flow cytometry of intracellular markers, and c) gene expression studies. These different methods, however, make comparisons of results difficult between studies of CFS patients. Some studies demonstrated abnormal release of IL- 1ß and IL-1Ra from PBMC [86], whereas others found no difference in IL-1ß in the peripheral blood of CFS patients [87]. Similarly, plasma levels of IL-1ß and IL-6 were initially found to be raised in CFS patients [88], but subsequently these results could not be corroborated [89, 90]. More recent reports demonstrate increased production of anti-inflammatory cytokines and reduced production of inflammatory cytokines by CFS patients [91]. Similar findings were reported in CFS patients with and without FM who demonstrated increased level of IL-10 compared to healthy controls during sleep [92]. These patients, however, had normal pro-inflammatory cytokines in their serum, peripheral blood lymphocytes mRNA or resting and stimulated peripheral blood leukocytes. In a large study of monozygotic (MZ) and dizygotic (DZ) twins discordant for CFS, the investigators could not detect differences between twins in serum levels of IL-4, IFN-γ and soluble CD23 as measured by ELISA [93, 94].

More recently, a new approach for identifying CFS abnormalities has focused on cytokine networks instead of individual cytokines mediating immune activity [95, 96]. Using this approach investigators measured several different cytokines, including IL-1, IL-4, IL- 6, IL-8, IL-10, IL-12, IL- 17, IFN-γ, and TNF-α in the plasma of CFS patients

Table 1. Cytokine abnormalities in patients with central sensitivity syndromes.

Diagnosis	Cytokines	Results	Author/Year
FM	IL-1ra	Increased compared to NC	Maes *et al*. 1999
	IL-1, IL-6, IL-8, IL-10, TNF	No abnormalities compared to NC	Bazzichi *et al*. 2007
	IL-4, IL-10	Decreased compared to NC	Uceyler *et al*. 2006
IBS	IL-6, IL-8, IL-10	Increased IL-6 compared to NC	Dinan *et al*. 2008
	IL-10, IL12	Increased compared to NC	O'Mahony *et al*. 2005
	IL-1, IL-6, IL-8, IL-12, IL-13	Increased IL-6 and IL-8	Scully *et al*. 2010
CFS	IL-1, IL-1ra	Increased compared to NC	Cannon *et al*. 1997
	TNF	Increased compared to NC	Patarka *et al*. 1994
	IL-4, IL-6, IL-10, IL-12, TNF, INF-y	No abnormalities compared to NC	Mawle *et al*. 1997
	IL-4, IL-6, IL-10, IL-12, TNF, INF-y	No abnormalities compared to NC	Natelson *et al*. 1999
	IL-1, INF-y, TNF	Increased compared to NC	Straus *et al*. 1989

and matched controls. They reported cytokine levels consistent with diminished T helper (Th) 1 and Th17 immune responses and also increased Th2 network activity [95]. Additionally, there was evidence for attenuated networks that contribute to NK cell activation and IL-12 activity.

However, a recent systematic review of 58 CFS studies demonstrated no clear differences in cytokine levels between CFS patients and normal controls [97]. Several studies assessing a wide range of cytokines in CFS patients did also not find any significant differences [65, 98]. In other studies, increased levels of TGF-β levels were inconsistently demonstrated [99, 100]. Overall, most cytokine abnormalities reported in CFS patients could not be consistently replicated in follow-up studies.

CYTOKINES IN IRRITABLE BOWEL SYNDROME

Approximately 15 percent of US adults report symptoms that are consistent with IBS [101]. This functional gastrointestinal disorder can lead to abdominal pain, cramping, and changes in bowel movements [102]. Diarrhea or constipation may predominate and similar to other CSS, chronic pain, fatigue, and psychological distress are frequently present, which often contribute to work absenteeism. Low-grade inflammation has been previously implicated in the pathophysiology of IBS [103] and imbalances of pro- and anti-inflammatory cytokines including polymorphisms of cytokine genes have been reported in this syndrome [104-106]. However, such findings have not been consistently observed. A recent meta-analysis showed no significantly different serum or plasma levels of TNF-α and IL-10 in IBS patients compared to controls [107]. A second meta-analysis investigated IL-10, TGF-ß1, and TNF-α gene polymorphisms in IBS patients and controls [108]. High producers of IL-10 were significantly associated with a decreased risk of IBS. However, no associations were found between TNF-α genotypes and IBS. Although this meta-analysis indicated a role for IL-10 polymorphisms in IBS it is currently unclear whether gene polymorphisms are associated with alterations in cytokine levels resulting in functional changes of the gut. Overall, these findings do not suggest a definitive role of cytokines in IBS and emphasize the need for more detailed evaluations in future studies.

CONCLUSION

The high degree of phenotypical overlap between FM, CFS, IBS and other somatically focused chronic pain syndromes has been conceptualized as CSS by Yunus due to the frequent presence of CS in patients with these illnesses [40]. However, CS is not a specific feature of CSS but has also been described in other chronic pain syndromes including peripheral neuropathy [109] and osteoarthritis [110, 111]. Cytokines have been a promising target of many CSS investigations because several are known to be pro-nociceptive and thus able to contribute to the development and maintenance of chronic pain. Specifically, cytokines have been thought to play an important etio-pathogenetic role in CFS because of its presumed association with chronic infections and immune deficiencies [112]. Although initial reports seemed to support the important role of specific cytokines for CFS and other CSS, subsequent studies often could not confirm these finding. It is possible, however, that some subgroups of patients with CSS may have cytokine driven abnormalities that explain their pain and other relevant symptoms. Currently, a major focus of research of cytokine signaling in CSS is directed towards abnormal contributions of glia in the peripheral and central nervous system which, at least in some animal models of chronic pain, appeared to hold great promise.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was funded in part by NIH grant R01 NR014049-01.

REFERENCES

[1] Dinarello CA. Historical insights into cytokines. Eur J Immunol 2007; 37: S34-S45.
[2] Bowen RA, Hortin GL, Csako G, *et al*. Impact of blood collection devices on clinical chemistry assays. Clin Biochem 2010; 43: 4-25.
[3] Bowen RA, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med (Zagreb) 2014; 24: 31-44.
[4] Keustermans GC, Hoeks SB, Meerding JM, *et al*. Cytokine assays: an assessment of the preparation and treatment of blood and tissue samples. Methods 2013; 61: 10-7.
[5] de Jager W, Bourcier K, Rijkers GT, *et al*. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC Immunol 2009; 10: 52-10.
[6] Parkitny L, McAuley JH, Kelly PJ, *et al*. Multiplex cytokine concentration measurement: how much do the medium and handling matter? Mediators Inflamm 2013; 2013: 890706: 890706.
[7] Skogstrand K, Ekelund CK, Thorsen P, *et al*. Effects of blood sample handling procedures on measurable inflammatory markers in plasma, serum and dried blood spot samples. J Immunol Methods 2008; 336: 78-84.
[8] Khan SS, Smith MS, Reda D, *et al*. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. Cytometry B Clin Cytom 2004; 61: 35-9.
[9] Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 2004; 361: 184-7.
[10] Uceyler N, Schafers M, Sommer C. Mode of action of cytokines on nociceptive neurons. Exp Brain Res 2009; 196: 67-78.
[11] Inui A. Cytokines and sickness behavior: implications from knockout animal models. Trends Immunol 2001; 22: 469-73.
[12] Staud R, Vierck CJ, Cannon RL, *et al*. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. Pain 2001; 91: 165-75.
[13] Staud R, Robinson ME, Price DD. Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients. J Pain 2007; 8: 893-901.
[14] Staud R. Abnormal pain modulation in patients with spatially distributed chronic pain: Fibromyalgia. Rheum Dis Clin North Am 2009; 35: 263-74.
[15] Staud R. Is it all central sensitization? Role of peripheral tissue nociception in chronic musculskeletal pain. Curr Rheumatol Rep 2010; 12: 448-54.
[16] Staud R. Objective biomarkers or symptom scores for the classification of fibromyalgia syndrome? Curr Rheum Rev 2012; 8: 307-17.
[17] Staud R, Weyl EE, Riley JL, III, *et al*. Slow temporal summation of pain for assessment of central pain sensitivity and clinical pain of fibromyalgia patients. PLoS One 2014; 9: e89086.
[18] McCrone P, Darbishire L, Ridsdale L, *et al*. The economic cost of chronic fatigue and chronic fatigue syndrome in UK primary care. Psychol Med 2003; 33: 253-61.
[19] Knight T, Schaefer C, Chandran A, *et al*. Health-resource use and costs associated with fibromyalgia in France, Germany, and the United States. Clinicoecon Outcomes Res 2013; 5: 171-80.
[20] Scholz J, Woolf CJ. The neuropathic pain triad: Neurons, immune cells and glia. Nat Neurosci 2007; 10: 1361-8.
[21] Ji RR, Berta T, Nedergaard M. Glia and pain: Is chronic pain a gliopathy? Pain 2013; 154: S10-S28.
[22] Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 2009; 32: 421-31.
[23] Marchand F, Perretti M, McMahon SB. Role of the immune system in chronic pain. Nature Reviews Neuroscience 2005; 6: 521-32.
[24] Bazzichi L, Rossi A, Massimetti G, *et al*. Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. Clin Exp Rheumatol 2007; 25: 225-30.
[25] Ross RL, Jones KD, Bennett RM, *et al*. Preliminary Evidence of Increased Pain and Elevated Cytokines in Fibromyalgia Patients with Defective Growth Hormone Response to Exercise. Open Immunol J 2010; 3: 9-18.
[26] Wang HL, Moser M, Schiltenwolf M, *et al*. Circulating cytokine levels compared to pain in patients with fibromyalgia - A prospective longitudinal study over 6 months. J Rheumatol 2008; 35: 1366-70.
[27] Lorusso L, Mikhaylova SV, Capelli E, *et al*. Immunological aspects of chronic fatigue syndrome. Autoimmunity Reviews 2009; 8: 287-91.
[28] Patarca R. Cytokines and chronic fatigue syndrome. Ann N Y Acad Sci 2001; 933: 185-200.
[29] Patarca R, Klimas NG, Lugtendorf S, *et al*. Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome: interrelations with cellular sources and patterns of soluble immune mediator expression. Clin Infect Dis 1994; 18 Suppl 1: S147-53.: S147-S153.
[30] Scully P, McKernan DP, Keohane J, *et al*. Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. Am J Gastroenterol 2010; 105: 2235-43.
[31] Clark AK, Old EA, Malcangio M. Neuropathic pain and cytokines: current perspectives. J Pain Res 2013; 6: 803-14.
[32] Wang XM, Hamza M, Wu TX, *et al*. Upregulation of IL-6, IL-8 and CCL2 gene expression after acute inflammation: Correlation to clinical pain. Pain 2009; 142: 275-83.
[33] Uceyler N, Valenza R, Stock M, *et al*. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. Arthritis and Rheumatism 2006; 54: 2656-64.
[34] Shah JP, Phillips TM, Danoff JV, *et al*. An *in vivo* microanalytical technique for measuring the local biochemical milieu of human skeletal muscle. J Appl Physiol 2005; 99: 1977-84.
[35] Shah JP. Uncovering the biochemical milieu of myofascial trigger points using *in vivo* microdialysis. J Musculoskelet Pain 2008; 16: 17-20.
[36] Gerdle B, Lemming D, Kristiansen J, *et al*. Biochemical alterations in the trapezius muscle of patients with chronic whiplash associated disorders (WAD) - A microdialysis study. Eur J Pain 2008; 12: 82-93.
[37] Rosendal L, Sogaard K, Kjaer M, *et al*. Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. J Appl Physiol 2005; 98: 477-81.
[38] Helmy A, Carpenter KL, Skepper JN, *et al*. Microdialysis of cytokines: methodological considerations, scanning electron microscopy, and determination of relative recovery. J Neurotrauma 2009; 26: 549-61.
[39] Yunus MB. Central Sensitivity Syndromes: A New Paradigm and Group Nosology for Fibromyalgia and Overlapping Conditions, and the Related Issue of Disease versus Illness. Semin Arthritis Rheum 2008; 37: 339-52.
[40] Yunus MB. Central Sensitivity Syndromes: An Overview. J Musculoskelet Pain 2009; 17: 400-8.
[41] Woolf CJ. Central sensitization: Implications for the diagnosis and treatment of pain. Pain 2011; 152: S2-S15.
[42] Woolf CJ. Windup and central sensitization are not equivalent. Pain 1996; 66: 105-8.
[43] Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009; 10: 895-926.
[44] Dickenson AH, Sullivan AF. NMDA receptors and central hyperalgesic states. Pain 1991; 46: 344-6.
[45] Wallace DJ, Linker-Israeli M, Hallegua D, *et al*. Cytokines play an aetiopathogenetic role in fibromyalgia: a hypothesis and pilot study. Rheumatology 2001; 40: 743-9.
[46] Dantzer R, Bluthe RM, Laye S, *et al*. Cytokines and sickness behavior. Ann N Y Acad Sci 1998; 840: 586-90.
[47] Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur J Pharmacol 2004; 500: 399-411.
[48] Kelley KW, Bluthe RM, Dantzer R, *et al*. Cytokine-induced sickness behavior. Brain Behav Immun 2003; 17: S112-S118.
[49] Caro XJ. Is there an immunologic component to the fibrositis syndrome? Rheum Dis Clin North Am 1989; 15: 169-86.
[50] Paiva ES, da Costa EDGM, Scheinberg M. Fibromyalgia: An update and immunological aspects. Curr Pain Headache Rep 2008; 12: 321-6.

[51] Littlejohn GO, Weinstein C, Helme RD. Increased neurogenic inflammation in fibrositis syndrome. J Rheumatol 1987; 14: 1022-5.

[52] Maes M, Libbrecht I, Van Hunsel F, *et al*. The immune-inflammatory pathophysiology of fibromyalgia: increased serum soluble gp130, the common signal transducer protein of various neurotrophic cytokines. Psychoneuroendocrinology 1999; 24: 371-83.

[53] Russell IJ, Orr MD, Littman B, *et al*. Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. Arthritis Rheumatol 1994; 37: 1593-601.

[54] Russell IJ. Neurochemical pathogenesis of fibromyalgia syndrome. J Musculoskel Pain 1996; 4: 61-92.

[55] Kadetoff D, Lampa J, Westman M, *et al*. Evidence of central inflammation in fibromyalgia - Increased cerebrospinal fluid interleukin-8 levels. J Neuroimmunol 2012; 242: 33-8.

[56] Wingenfeld K, Heim C, Schmidt I, *et al*. HPA axis reactivity and lymphocyte glucocorticoid sensitivity in fibromyalgia syndrome and chronic pelvic pain. Psychosomatic Medicine 2008; 70: 65-72.

[57] Gur A, Oktayoglu P. Status of immune mediators in Fibromyalgia. Curr Pain Headache Rep 2008; 12: 175-81.

[58] Dunn AJ. Cytokine activation of the HPA axis. Ann N Y Acad Sci 2000; 917: 608-17.

[59] Malcangio M, Bowery NG, Flower RJ, *et al*. Effect of interleukin-1 beta on the release of substance P from rat isolated spinal cord. Eur J Pharmacol 1996; 299: 113-8.

[60] Takahashi S, Kapas L, Fang J, *et al*. Somnogenic relationships between tumor necrosis factor and interleukin-1. Am J Physiol 1999; 276: R1132-R1140.

[61] Wallace DJ. Is there a role for cytokine based therapies in fibromyalgia. Curr Pharm Design 2006; 12: 17-22.

[62] Papanicolaou DA, Wilder RL, Manolagas SC, *et al*. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 1998; 128: 127-37.

[63] Uceyler N, Hauser W, Sommer C. Systematic review with meta-analysis: cytokines in fibromyalgia syndrome. BMC Musculoskeletal Disorders 2011; 12.

[64] Uceyler N, Valenza R, Stock M, *et al*. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. Arthritis Rheumatol 2006; 54: 2656-64.

[65] Kashipaz MRA, Swinden D, Todd I, *et al*. Normal production of inflammatory cytokines in chronic fatigue and fibromyalgia syndromes determined by intracellular cytokine staining in short-term cultured blood mononuclear cells. Clin Exp Immunol 2003; 132: 360-5.

[66] Bansal AS, Bradley AS, Bishop KN, *et al*. Chronic fatigue syndrome, the immune system and viral infection. Brain Behav Immun 2012; 26: 24-31.

[67] Reid S, Chalder T, Cleare A, *et al*. Chronic fatigue syndrome. Clin Evid 2002;1075-88.

[68] Afari N, Buchwald D. Chronic fatigue syndrome: a review. Am J Psychiatry 2003; 160: 221-36.

[69] Komaroff AL, Cho TA. Role of Infection and Neurologic Dysfunction in Chronic Fatigue Syndrome. Seminars in Neurology 2011; 31: 325-37.

[70] Komaroff AL, Buchwald D. Symptoms and signs of chronic fatigue syndrome. Rev Infect Dis 1991; 13 Suppl 1: S8-11.

[71] Christley Y, Duffy T, Martin CR. A review of the definitional criteria for chronic fatigue syndrome. Journal of Evaluation in Clinical Practice 2012; 18: 25-31.

[72] Reid S, Chalder T, Cleare A, *et al*. Chronic fatigue syndrome. BMJ 2000; 320: 292-6.

[73] Patarca-Montero R, Antoni M, Fletcher MA, *et al*. Cytokine and other immunologic markers in chronic fatigue syndrome and their relation to neuropsychological factors. Appl Neuropsychol 2001; 8: 51-64.

[74] Stewart CC, Cookfair DL, Hovey KM, *et al*. Predictive immunophenotypes: Disease-related profile in chronic fatigue syndrome. Cytometry 2003; 53B: 26-33.

[75] Brenu EW, Hardcastle SL, Atkinson GM, *et al*. Natural killer cells in patients with severe chronic fatigue syndrome. Autoimmune Highlights 2013; 4: 69-80.

[76] Huth TK, Brenu EW, Nguyen T, *et al*. Characterization of natural killer cell phenotypes in chronic fatigue syndrome/myalgic encephalitis. Clin Cell Immunol 2014; 5: 1-8.

[77] Maes M, Twisk FNM, Kubera M, *et al*. Evidence for inflammation and activation of cell-mediated immunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): Increased interleukin-1, tumor necrosis factor-alpha, PMN-elastase, lysozyme and neopterin. J Affect Disord 2012; 136: 933-9.

[78] Stringer EA, Baker KS, Carroll IR, *et al*. Daily cytokine fluctuations, driven by leptin, are associated with fatigue severity in chronic fatigue syndrome: evidence of inflammatory pathology. Journal of Translational Medicine 2013; 11.

[79] Anderson G, Berk M, Maes M. Biological phenotypes underpin the physio-somatic symptoms of somatization, depression, and chronic fatigue syndrome. Acta Psychiatr Scand 2013;10.

[80] Chia J, Chia A, Voeller M, *et al*. Acute enterovirus infection followed by myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and viral persistence. J Clin Pathol 2010; 63: 165-8.

[81] Kerr JR. Enterovirus infection of the stomach in chronic fatigue syndrome/myalgic encephalomyelitis. J Clin Pathol 2008; 61: 1-2.

[82] Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 2007; 21: 153-60.

[83] Buchwald D, Wener MH, Pearlman T, *et al*. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J Rheumatol 1997; 24: 372-6.

[84] Moss RB, Mercandetti A, Vojdani A. TNF-alpha and chronic fatigue syndrome. J Clin Immunol 1999; 19: 314-6.

[85] Raison CL, Lin JMS, Reeves WC. Association of peripheral inflammatory markers with chronic fatigue in a population-based sample. Brain Behavior and Immunity 2009; 23: 327-37.

[86] Cannon JG, Angel JB, Abad LW, *et al*. Interleukin-1 beta, interleukin-1 receptor antagonist, and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. J Clin Immunol 1997; 17: 253-61.

[87] Nijs J, Van Oosterwijck J, Meeus M, *et al*. Unravelling the nature of postexertional malaise in myalgic encephalomyelitis/chronic fatigue syndrome: the role of elastase, complement C4a and interleukin-1beta. J Intern Med 2010; 267: 418-35.

[88] Fletcher MA, Zeng XR, Barnes Z, *et al*. Plasma cytokines in women with chronic fatigue syndrome. Journal of Translational Medicine 2009; 7.

[89] Nater UM, Youngblood LS, Jones JF, *et al*. Alterations in diurnal salivary cortisol rhythm in a population-based sample of cases with chronic fatigue syndrome. Psychosomatic Medicine 2008; 70: 298-305.

[90] Natelson BH, Denny T, Zhou XD, *et al*. Is depression associated with immune activation? J Affect Disord 1999; 53: 179-84.

[91] ter Wolbeek M, van Doornen LJP, Kavelaars A, *et al*. Longitudinal analysis of pro- and anti-inflammatory cytokine production in severely fatigued adolescents. Brain Behavior and Immunity 2007; 21: 1063-74.

[92] Nakamura T, Schwander SK, Donnelly R, *et al*. Cytokines across the Night in Chronic Fatigue Syndrome with and without Fibromyalgia. Clinical and Vaccine Immunology 2010; 17: 582-7.

[93] Hickie IB, Bansal AS, Kirk KM, *et al*. A twin study of the etiology of prolonged fatigue and immune activation. Twin Res 2001; 4: 94-102.

[94] Hickie I, Lloyd A, Hadzi-Pavlovic D, *et al*. Can the chronic fatigue syndrome be defined by distinct clinical features? Psychol Med 1995; 25: 925-35.

[95] Broderick G, Fuite J, Kreitz A, *et al*. A formal analysis of cytokine networks in chronic fatigue syndrome. Brain Behav Immun 2010; 24: 1209-17.

[96] Hornig M, Gottschalk G, Peterson DL, *et al*. Cytokine network analysis of cerebrospinal fluid in myalgic encephalomyelitis/chronic fatigue syndrome. Mol Psychiatry 2015;1-9.

[97] Lyall M, Peakman M, Wessely S. A systematic review and critical evaluation of the immunology of chronic fatigue syndrome. J Psychosom Res 2003; 55: 79-90.

[98] Mawle AC, Nisenbaum R, Dobbins JG, *et al*. Immune responses associated with chronic fatigue syndrome: a case-control study. J Infect Dis 1997; 175: 136-41.

[99] Chao CC, Janoff EN, Hu SX, *et al*. Altered cytokine release in peripheral blood mononuclear cell cultures from patients with the chronic fatigue syndrome. Cytokine 1991; 3: 292-8.

[100] Bennett AL, Chao CC, Hu S, *et al*. Elevation of bioactive transforming growth factor-beta in serum from patients with chronic fatigue syndrome. J Clin Immunol 1997; 17: 160-6.

[101] Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol 2012; 10: 712-21.

[102] Horwitz BJ, Fisher RS. The irritable bowel syndrome. N Engl J Med 2001; 344: 1846-50.

[103] Barbara G, Cremon C, Carini G, *et al*. The immune system in irritable bowel syndrome. J Neurogastroenterol Motil 2011; 17: 349-59.

[104] Bashashati M, Rezaei N, Andrews CN, *et al*. Cytokines and irritable bowel syndrome: where do we stand? Cytokine 2012; 57: 201-9.

[105] O'Mahony L, McCarthy J, Kelly P, *et al*. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 2005; 128: 541-51.

[106] Dinan TG, Clarke G, Quigley EM, *et al*. Enhanced cholinergic-mediated increase in the pro-inflammatory cytokine IL-6 in irritable bowel syndrome: role of muscarinic receptors. Am J Gastroenterol 2008; 103: 2570-6.

[107] Bashashati M, Rezaei N, Shafieyoun A, *et al*. Cytokine imbalance in irritable bowel syndrome: a systematic review and meta-analysis. Neurogastroenterol Motil 2014; 26: 1036-48.

[108] Bashashati M, Rezaei N, Bashashati H, *et al*. Cytokine gene polymorphisms are associated with irritable bowel syndrome: a systematic review and meta-analysis. Neurogastroenterol Motil 2012; 24: 1102-e566.

[109] Nickel FT, Seifert F, Lanz S, *et al*. Mechanisms of neuropathic pain. Eur Neuropsychopharmacol 2012; 22: 81-91.

[110] Murphy SL, Phillips K, Williams DA, *et al*. The Role of the Central Nervous System in Osteoarthritis Pain and Implications for Rehabilitation. Current Rheumatology Reports 2012; 14: 576-82.

[111] Lluch E, Torres R, Nijs J, *et al*. Evidence for central sensitization in patients with osteoarthritis pain: A systematic literature review. Eur J Pain 2014; 18: 1367-75.

[112] Brenu EW, van Driel ML, Staines DR, *et al*. Immunological abnormalities as potential biomarkers in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. J Transl Med 2011; 9: 81: 81.

Received: May 20, 2015 Revised: June 10, 2015 Accepted: June 11, 2015