Chapter

Chemokines as Potential Biomarkers for PTSD in Military Population

Lei Zhang, Xianzhang Hu, Xiaoxia Li and Robert J. Ursano

Abstract

Post-traumatic stress disorder (PTSD) is a serious mental health concern worldwide among civilians and military personnel. Gaps in our understanding of its biological basis create significant obstacles for accurate diagnosis and assessment of therapeutic interventions. In light of this, investigation of biological factors associated with possible molecular cues of inflammation or neuroimmune disorders, could provide new surrogate markers for PTSD or PTSD treatment response. Analyses to date in deployed military personnel have suggested that sets of chemokines may be useful as biomarkers for PTSD acquired in military operations. Specifically, studies to date suggest that CCL2, CCL15, CCL22, CCL25, CXCL2, and CXCL12 are associated with PTSD onset, while CCL13, CCL20, and CXCL6 are correlated to PTSD risk; CX3CL1 are associated with resilience; CCL3; CXCL11, and CXCL16 are associated with stress response. CCL11, CCL13, CCL20, and CCL25 are correlated with the severity of PTSD symptoms. This chapter reviews the current understanding of potential chemokine markers for PTSD, and the potential chemokines associated with PTSD onset, risk, resilience, as well as stress responses in service members. Although the proposed biomarkers require further validation, these findings may lead to additional knowledge for the education and development of diagnostic and therapeutic approaches for PTSD, not only benefiting military personnel, but civilians as well.

Keywords: Post-traumatic stress disorder, PTSD, cytokine, chemokine, biomarker

1. Introduction

Post-traumatic stress disorder (PTSD) is a stress related disorder. Its lifetime prevalence in U.S. general population is about 7% to 9% and the point prevalence of PTSD in combat veterans is 17% [1]. During the Iraq and Afghanistan Wars, the point prevalence was significantly increased in the military population. About 10–18% of service members manifest probable PTSD following deployment (https://www.ptsd.va.gov/public/PTSD-overview/basics/how-common-is-ptsd.asp). The neuropsychological impairments of PTSD can be debilitating, and significantly affect mental and physical function. Cognitive-behavioral psychotherapies are used to treat patients [2–4]. There are only two US Food and Drug Administration (FDA) approved medications for PTSD, sertraline and paroxetine, which are selective serotonin reuptake inhibitor antidepressants [5]. However, their therapeutic efficacy is not sufficient in some patients. Moreover, the molecular mechanisms underlying PTSD remain largely unknown. Presently, PTSD diagnosis

is based on clinical history, mental status, symptom duration, and symptom checklists or patient self-reports and lack of objective biomarker tests. To begin early intervention, objective diagnostic approaches are needed. Progress is being make [6]. Thus, a diagnostic biomarker test in the early stage or a treatable stage of the disorder would be beneficial for physicians and patients.

Biomarker research has been incrementally translated into clinical applications and has provided the necessary platform for development of novel therapeutic [7] and diagnosis approaches. One emerging area in PTSD research is the association studies regarding central nervous system (CNS)-specific immune proteins such as cytokines and chemokines. Several studies have demonstrated that cytokines play a role in brain development and function, and affect the neural circuits and transmitters within the brain, causing changes in behavior. The levels of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-alpha, and C-reactive protein (CRP), are linked to stress related disorders such as major depression [8] and PTSD.

The association of PTSD with a pro-inflammatory activation of the immune system may contribute to accelerated aging [9]. PTSD has shown an association with aging, cardiovascular disease [10] autoimmune disorders and dementia [11]. In this chapter we review the reports from the translational studies about chemokines in PTSD of military service members. The translational research provides not only a better understanding of the molecular mechanisms of the devastating stress–related disorders, but also the knowledge for developing a possible diagnostic approach beneficial to both civilians and military service members.

2. Cytokine and stress-related disorders, PTSD and depression

Substantial evidence demonstrates that PTSD is associated with cytokine dysfunction. For example, patients with PTSD have higher serum cytokine concentrations than those without PTSD in anti-CCP positive rheumatoid arthritis (RA) subjects [12]. PTSD has significantly higher levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α peripheral blood mononuclear cells (PBMCs). The elevated levels are also associated with PTSD symptom severity [13].

Increased levels of cytokines may activate some neurotransmitter pathways reducing growth factor concentrations while producing changes in monoamines, glutamate, and other peptide transmitters. The cytokines themselves may be the result of environmental factors or psychiatric stress such as trauma during childhood, current stress, sleep disorder. Chronic stress results in upregulated cytokines, which dysregulate the neurotransmitter and cellular signal transduction leading to depression and other diseases [14]. Cytokines are able to go through the brain-barrier [15] Peripheral cytokine levels may reflect central action and be associated with symptoms of depression and anxiety [16]. Depressed patients without medical illness and who respond poorly to antidepressants have higher circulating inflammatory cytokine levels. Their traumatic childhood experiences may play a role in their later chronic inflammation and depressive condition. The association of pro-inflammatory cytokines with degenerative processes indicates that depressive disorders may have or enhance analogous functions in the systemic immune system [17, 18].

3. PTSD and chemokines in service members

A recent study found that chemokines, a family of small cytokines [19] were associated with stress-related disorders including PTSD [12, 20]. Chemokines

are chemotactic factors regulating the migration of peripheral immune cells – an action, likely relevant to the pro-inflammatory cascades [19]. In addition to chemotaxis, chemokines potentiate and activate peripheral immune cells to direct the pro-inflammatory activation state, contributing to the neurodegenerative and pro-apoptotic cascades often seen in depression and Alzheimer's disease [21–24]. For example, CXCL11 is associated with aging-related impairment, including impairment of hippocampal neurogenesis, learning and memory [25]. Early evidence suggests novel non-immune and CNS-specific mechanisms of chemokines, including neuromodulation, neuroendocrine regulation, and direct neurotransmitter-like actions [26–28]. Moreover, an animal study shows that chemokine receptor (CCR6, CCR7, CXCR5) knockout mice demonstrate psychiatric- and neurobiological- like behaviors [29–31].

Chemokines regulate leukocyte migration and positioning though their receptors, which are expressed on the target cell surface [32]. Their molecular weight is 8–13 kDa. Their receptors are typical G protein-coupled transmembrane proteins, which may bind multiple ligands with variable affinity [19, 33]. There are about 44 chemokines, which are categorized into four different families (CC, CXC, CX3C, C) according to their biological behavior and structure. They regulate intracellular signaling leading to increased intracellular calcium [34] and may directly interact with G-protein-coupled receptors [35]. They also regulate the release of pro-inflammatory mediators and control of T-helper (Th)-1/Th-2 phenotypic polarization [33, 36]. Their receptor expression is observed in the brain [37–40]. Certain chemokines, such as CCL2, CCL3, CCL19, CCL21, CXCL8, CXCL12, and CX3CL1, are expressed under physiological conditions [40], while other may be expressed and upregulated in response to injury or inflammation.

The diversity of accordance and severity of PTSD symptoms following exposure to traumatic events has been a challenge for PTSD research. The mechanism underlying the diversity of PTSD symptoms with the passage of time involves a complicated course including stress response and resilience. This perspective has important implications for chemokine marker research, which is related to not only the underlying PTSD pathology, but also the dysregulation and abnormalities of immune function following exposure to traumatic stress. Recently, a prospective cohort study (paired and non-paired, pre- and post-deployment design) found [41] seven dysregulated chemokines biomarkers for PTSD, including 5CC (CCL2, CCL15, CCL23, CCL22 and CCL25,) and 2 CXC (CXCL2 and CXCL12), five possible PTSD risk markers, four stress response markers and one resilience marker (**Figure 1**).

As disease biomarkers for PTSD, 5CC (CCL2, CCL15, CCL23, CCL22 and CCL25,), and 2 CXC (CXCL2 and CXCL12) have been found dysregulated. Among them, CCL2, CCL22, CCL15, and CXCL2 were significantly upregulated, while CCL25 and CXCL12 were downregulated, in subjects with PTSD.

Considering stress response, potential chemokine markers response include CCL3 and CXCL5 which have been shown to be downregulation, while the markers CXCL 11 and CXCL16 have been shown to be up-regulated. Moreover, as risk markers for PTSD, CCL13, CCL23 and CXCL6 levels were lower pre-deployment in solders who developed PTSD than in control pre-deployment. But, CCL20 was significantly higher in the case of pre- deployment than in the control pre-deployment soldiers. Differences of CCL23 levels were also identified between PTSD pre-deployment and PTSD post-deployment, indicating there is an overlap of CCL23 dysregulation among the control, non-PTSD, case control and case. Therefore, it may be a "sticky" marker for subjects at PTSD risk or with PTSD.

CX3CL1 has been identified as a possible resilience marker (comparing the levels of chemokines between PTSD at post-deployment and non-PTSD of

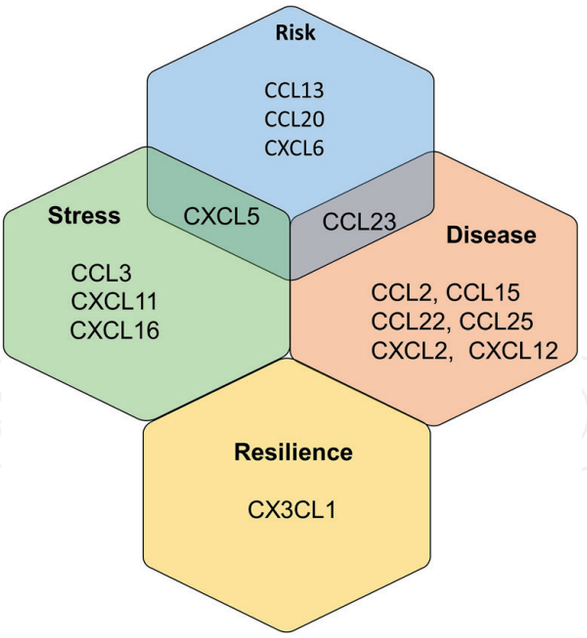

Figure 1.
Venn diagram showing potential chemokine markers for PTSD occurrence, risk, and resilience, as well as stress response. The overlap of two potential chemokine markers (CXCL5 and CCL23) is also shown.

post-deployment). CX3CL1 was not significantly different between controls at pre-deployment and controls at post-deployment. The data indicate that although deployment resulted in an up-regulation of CX3CL1 in soldiers with PTSD prior to deployment (case pre- vs. case post-), it did not alter the basal levels of CX3CL1 in non-PTSD controls (control pre- vs. control post-). Therefore CX3CL1 may be a potential resilience marker [20].

The four subgroups of chemokines are listed in **Table 1**: CXC, CC, CX3C and C. There are two adjacent cysteines (amino acids), near their amino terminus in the CC chemokine (or β-chemokine) proteins. The two N-terminal cysteines of CXC chemokines (or α-chemokines) are separated by one amino acid, represented in this name with an "X". Unlike all other chemokines C chemokines have only two cysteines; one N-terminal cysteine and one cysteine downstream. A fourth group CX3C has three amino acids between the two cysteines. CX3CL1 is only CX3C chemokine discovered. It is a chemoattractant and serves as an adhesion molecule. **Table 1** shows the summary of subgroup of chemokines which are potential markers for PTSD: onset, risk and resilience as well as stress response. **Figure 2** demonstrates subgroups of chemokine as different markers. These results warrant further systematic analytical and clinical validation research.

Dysregulation of the immune system in the stress response has long been considered a remarkable abnormal physiologic process in PTSD and stress associated disorders in service members and veterans [42, 43]. For example veterans coming back from the Gulf War have been reported to be more likely to suffer from rheumatism [44], sarcoidosis and multiple sclerosis suggesting that immunological changes may be involved in the occurrence and maintenance of PTSD (https://www.livescience.com/8916-battle-7-health-problems-facing-veterans.html). Cytokine/chemokine biomarkers have also been specifically associated with Gulf War Illness (GWI) in military population [45–47]. In a provocative study of PBMCs of chronic fatigue syndrome (CFS) subjects, over 60% of the CFS patients had DNA from a human gammaretrovirus, xenotropic murine leukemia virus-related virus (XMRV) while only 3.7% were identified in controls. Moreover, patient-derived XMRV was infectious [47], indicating it is possible that XMRV may be a contributing factor in the immune responses or pathogenesis of CFS. In addition, it has also

Markers	PTSD	Stress response	PTSD risk	Resilience
Chemokines				
CC	CCL2	CCL3	CCL13	
	CCL15		CCL20	
	CCL22		CCL23	
	CCL23			
	CCL25			
CXC	CXCL2	CXCL5	CXCL5	
	CXCL12	CXCL11	CXCL6	
		CXCL16		
CX3C				CX3CL1
C				

Table 1.
Summary of subgroups of potential chemokine markers for PTSD, PTSD risk and resilience, as well as stress response.

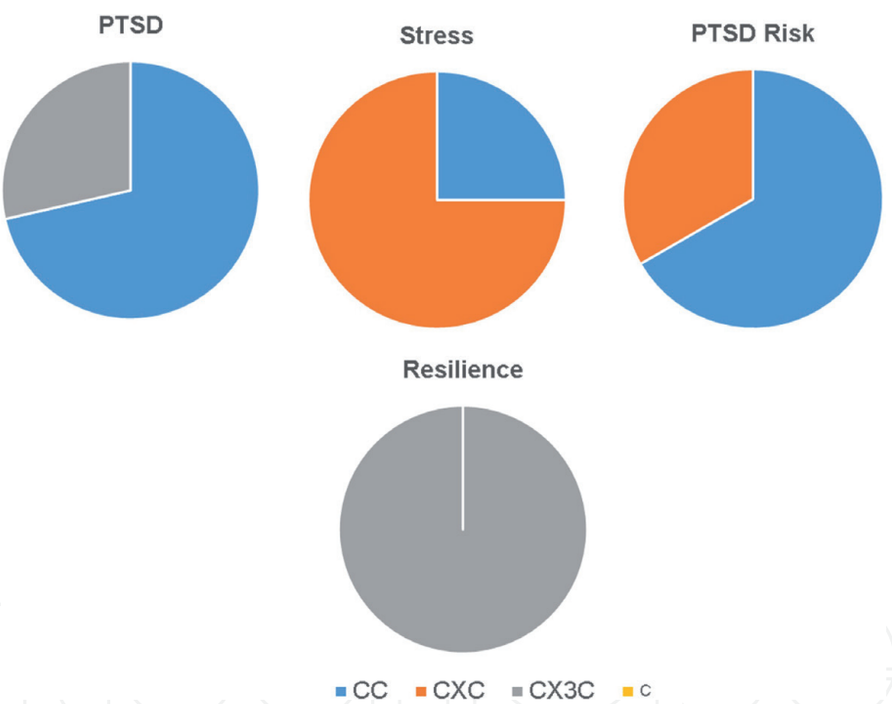

Figure 2.
Distribution of subgroups of chemokines as different markers as found in our previous study. Findings suggest that 15 out of 40 chemokines are differentially associated with PTSD, PTSD risk, stress-responses and resilience.

been reported that GWI patients had higher lymphocyte, monocyte, neutrophil, and platelet counts compared with controls. The six serum proteins of inflammation were also significantly different from controls including C reactive protein [45]. The results suggest that inflammation is a component of the pathobiology of GWI in the veterans.

Some studies have suggested that the risk of autoimmune disorders in PTSD is higher in psychiatric subjects than in those with no psychiatric diagnosis [48]. Interferons (INF)s, interleukins (ILs), lymphokines, tumor necrosis factor (TNF), which are often altered in response to an immune stimulus are involved in regulation of immunity and inflammation [9–11]. PTSD may have higher levels of certain cytokines (IL-2, IL-4, IL-6, IL-8, IL-10 and TNF-α) than age- and gender-matched

healthy controls, suggesting a generalized inflammatory state in PTSD [49]. Experimental endotoxin injection up-regulates plasma IL6, IL-10, and TNF-α, which has been associated with depression-like symptoms often seen in PTSD [50]. However, the results of cytokine levels in PTSD are mixed. While some reports show that PTSD patients had higher levels of IL-1β [51], IL-6, and TNF-α in the plasma [52], other reports no significant difference in levels of IL-1β [53] and IL-6 [53, 54] and IL-8 [54] between PTSD and controls [55]. These inconsistent findings may be partially attributable to variation of study subjects including differences of traumatic events (e.g. childhood vs. adulthood), the trauma duration (e.g. Life time vs. current), samples from different population (general population vs. service members), differences of geography and treatment (with or without), and testing approaches (ELISA vs. Western blot, and blood vs. saliva or CSF).

In our recent chemokine study, we used a prospective study design and collected PCL scores and blood samples from US soldiers pre- and post-deployment (pre-, post-) to a war zone during the Iraq and Afghanistan. We examined multiple (40) chemokines using luminex assay (a high-throughput biochip). All subjects experienced deployment to the war. The controls were not only self- but also age- and sex-matched. All subjects were at the same location (Guam) and was also Reserve or National Guard who had no medication. Our hypothesis we tested was that deployment stressful life events area associated with PTSD and chemokine/cytokine biomarkers. Potential disease markers, the blood chemokines, showed significant differences between PTSD cases pre- and post-; different basal levels of blood chemokine between non-PTSD control pre- and PTSD cases pre- being the risk markers for PTSD; different chemokine levels between non-PTSD controls pre- and non-PTSD controls post- may be possible stress response markers, and the differences between PTSD cases post- and non-PTSD controls post representing possible markers for resilience.

In recent years, cytokine detection and quantification has served as an important tool in biomarker research due to the capacity of simultaneous measurements of multiple cytokines in a single run in a small sample size. Simultaneous measurements of multiple blood cytokines provide an experimental strategy resolving complex interactions among signaling molecules to obtain a pattern of numerous cytokines within an experiment. It may also provide a more inclusive and comprehensive depiction of the association of cytokines with PTSD. It appears to be better than conventional assays such as ELISA, which measures individual cytokine only [20]. In addition, a prospective research design is important [41]. These factors make the study of chemokines/cytokines more significant for determining (their association with PTSD).

4. Concluding remarks

Although studies show cytokines and chemokines are associated with stress-related conditions, such as depression and PTSD particularly in the military population, the results sometimes are inconclusive. The study of chemokine biomarkers for PTSD is still in its early stages. Current studies suggest there is an excess of inflammatory action of the immune system in PTSD, which is linked to PTSD vulnerability. It is possible that dysregulation of cortisol plays an important role in this excessive inflammation. Moreover, accumulating evidence both at the bench and in the clinic indicate that dysregulated chemokines are potential molecular targets for diagnosis and treatment of PTSD. Potential chemokine biomarkers associated with PTSD onset, risk and resilience as well as other stress responses have been identified in military population (**Figure 1**). Translational research in service members

leads some support to the idea that altered chemokine expression may contribute to PTSD pathophysiology. This may in turn provide an opportunity to identify chemokine biomarkers not only for PTSD onset, risk, and resilience, but also other stress-responses as well.

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Disclaimer statement

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