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A Systematic Review of Tumor Necrosis Factor-α in Post-Traumatic Stress Disorder: Evidence from Human and Animal Studies

(Short Title: Systematic review of TNF-α and PTSD)

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Abstract

Background: Growing evidence suggests a pathophysiological role of cytokines in post-traumatic stress disorder (PTSD). Tumor necrosis factor (TNF)-α is a key cytokine. Therefore, we performed a systematic review to examine the findings regarding TNF-α derived from both animal and human studies of PTSD.

Methods: Using PRISMA guidelines, we reviewed relevant articles in PubMed from inception until 11th April 2017. Human studies that reported group comparisons and/or longitudinal investigations of TNF-α production/concentration were included. Research reporting on TNF-α levels in animal models of PTSD were also included.

Results: Twenty-seven articles were identified. Data from human cross-sectional studies suggests that plasma/serum levels of TNF-α are elevated in those with PTSD, as compared to healthy controls. Longitudinal assessments of TNF-α are limited and data are mixed. Limited data from animal studies suggest an increased TNF-α production in the hippocampus of rats following stress, which can be reversed by immunomodulatory drugs.

Conclusions: Our findings suggest TNF-α may be a potential biomarker and treatment target for PTSD. Findings need to be considered in light of heterogeneous methods for measurement and analysis of TNF-α concentration. Longitudinal research is needed to understand the role of TNF-α in the development and/or maintenance of PTSD.

Key Words: Cytokines, Tumor necrosis factor (TNF)-alpha, Post-traumatic stress disorder, Biomarker, Immunomodulatory drugs, Cytokines

Introduction

Post-traumatic stress disorder (PTSD) is a mental disorder, which may develop following an exposure to traumatic events, such as war, catastrophic accidents, and instances of violence. According to the International Classification of Diseases (ICD)-10, the specific criteria for diagnosis include (i) the exposure to a stressful event or situation of exceptionally threatening or catastrophic nature; (ii) a persistent remembering or "reliving" of the stressor, and (iii) avoidance of circumstances resembling or associated with the stressor. In addition, either the inability to recall important aspects of the period of exposure to the stressor, or persistent symptoms of increased psychological sensitivity and arousal need to be present (WHO 1992). To make the diagnosis, these symptoms must persist for more than a month after the occurrence of a traumatic event (WHO 1992). Similar criteria are used in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 to diagnose PTSD, namely the exposure to a traumatic event, in addition to having symptoms from each of the four symptom clusters: avoidance, intrusion, negative alterations in cognitions and mood, and alterations in arousal and reactivity. Furthermore, the functioning of a patient must be impaired and their symptoms should not be attributable to substance abuse or a co-occurring medical condition (APA 2013). To establish the diagnosis of PTSD, several clinical interviews and questionnaires are available, most of which are based on DSM-IV or DSM-5 criteria. Examples of diagnostic instruments related to the presented topic are the Clinician-Administered PTSD Scale (CAPS; Blake et al. 1995), the PTSD Checklist (PCL; Blanchard et al. 1996; a civilian and military version are also available, PCL-C and PCL-M respectively, Weathers et al. 2013), the Structured Clinical Interview for DSM Disorders (SCID-I; Spitzer et al. 1992, Ventura et al. 1998), Los Angeles Symptom Checklist (LASC; King et al. 1995), the Short Post-Traumatic Stress Disorder Rating Interview (SPRINT; Connor & Davidson 2001) and the Posttraumatic Diagnostic Scale (PDS; Foa et al. 1997, Sheeran & Zimmerman 2002).

Traumatic exposure itself is not the only causal factor for PTSD. The type and duration of trauma, psychological factors like personality characteristics, gender, and biological factors all seem to play a significant role in PTSD aetiology (Yehuda et al. 1995, Breslau et al. 1997, Frans et al. 2005). Several mechanisms have been implicated in the pathophysiology of PTSD including genetics, epigenetics, and neurobiological systems (Pitman et al. 2012, Bailey et al. 2013, Ryan et al. 2016). Recently, PTSD research has focused on immune mechanisms, for example, peripheral blood mononuclear cells (PBMCs) and cytokines, such as tumor necrosis factor - alpha (TNF-α; Andrews & Neises 2012). TNF-α is an inflammatory, pleiotropic cytokine mainly produced by macrophages to aid immune cell regulation (Lebrec et al. 2015). Apart from its key immune-regulatory role, it has been associated with several psychiatric disorders including depression, schizophrenia, and Alzheimer's disease (Himmerich et al. 2008, Lorz et al. 2009, Schmidt et al. 2014, Balõtšev et al. 2016, Decourt et al. 2017). Furthermore, research has suggested pro-inflammatory markers, such as TNF-α, are potentially involved in the development of PTSD (von Känel et al. 2007). TNF-α acts via two types of TNF receptors (TNF-R), TNF-R p55 and TNF-R p75. TNF-α is produced from peripheral immune cells and also in the central nervous system, having receptors on the surface of neurons and glial cells (Idriss & Naismith 2000). Evidence suggests that the blood-brain barrier is permeable to peripherally produced cytokines, including TNF-α. Thus, TNF-α can influence brain physiology by stimulating the hypothalamic-pituitary-adrenocortical (HPA) axis, activating monoamine reuptake and decreasing production of serotonin, due to the increased activity of indolamine-2,3-dioxygenase (Lichtblau et al. 2013).

Growing evidence suggests a pathophysiological role of pro-inflammatory cytokines in PTSD. As TNF-α is a key cytokine of the immune response (Lebrec et al. 2015), for which blocking

drugs are available, we sought to carry out this review of research investigating TNF-α concentrations and/or production in human and animal studies of PTSD. The purpose of this systematic review is to summarise the evidence regarding the role of TNF-α in PTSD, and to assess its potential as a biomarker for PTSD and its treatment, in addition to assessing it as a potential future drug target. Specifically, we aim to (i) determine whether TNF-α concentration/production differs between those with and without PTSD; (ii) assess whether TNF-α concentrations change over time and/or in response to treatment; and (iii) explore the role of TNF-α in animal models of PTSD.

Methods

This systematic review was conducted following the recommendations outlined in the PRISMA guidelines (Moher et al. 2009).

Selection Criteria

Studies of any design that assessed TNF-α production *in-vitro* or TNF-α concentration in the serum, plasma, or cerebrospinal fluid (*in-vivo*) of individuals with PTSD were eligible for inclusion. Studies were included if they reported a group and/or longitudinal comparison of TNF-α concentration/production. Publications reporting on the measurement of TNF-α, the TNF-α protein or its messenger ribonucleic acid (mRNA) in animal models of PTSD were also included.

Studies were excluded if: (i) they focused on life difficulties or trauma rather than PTSD as defined by ICD-10 and DSM-5; (ii) they reported genetic data only; (ii) they measured TNF receptors only; (iv) they were investigating glucocorticoid receptor (GR) sensitivity only; or (v) they did not report a group and/or longitudinal comparison of TNF-α concentration/production. Review articles, meta-analyses, conference proceedings/abstracts, editorials, letters, book chapters, and unpublished theses were also not included.

Search Strategy

Pubmed was searched from inception until the 11th April 2017 using the following key search terms: (("post-traumatic stress disorder"[Title/Abstract]) OR ("PTSD"[Title/Abstract])) AND (("tumor necrosis factor-alpha"[Title/Abstract]) OR ("tumor necrosis factor"[Title/Abstract]) OR ("TNF-alpha"[Title/Abstract]) OR ("TNF"[Title/Abstract]))

This search was supplemented by internet searches and hand-searches of reference lists of included papers and potentially relevant reviews. Citation tracking in Google Scholar was also performed. Identified articles had their titles and abstracts screened according to the pre-specified eligibility criteria. The eligible articles were further reviewed in full text. The articles were subsequently categorized into animal and human studies and then further divided according to their study design. An overview of the literature search is shown in Figure 1.

Data Extraction

The data from all included studies was extracted into an electronic summary table by the first author (SM), which was then checked by another author (BD). Information collected related to the sample characteristics, study design, and relevant findings.

Figure 1 here

Results

Characteristics of included studies

A total of 27 articles were eligible for inclusion in this review. We identified three articles that used animal models of PTSD to assess TNF-α and 24 human studies (including data from a total of 1865 participants). The human studies were further categorized into articles with cross-sectional data using *in vivo* methods (n=19; including studies measuring serum or plasma concentrations of TNF-α, articles with cross-sectional data on TNF-α production *in-vitro* (n=5), or articles with longitudinal data (n=3), two of which considered the effect of treatment interventions on TNF-α concentrations. One of the included studies reported both cross-sectional and longitudinal data (Jergović et al. 2015) and another study provided data on *in-vivo* and *in-vitro* measurements of TNF-α (Gola et al. 2013).

Assessment of PTSD symptoms. Various questionnaires were used for assessment of PTSD symptoms in the included studies. Most studies used the CAPS (e.g. von Känel et al. 2007, Hammad et al. 2012, Gola et al. 2013, Lindqvist et al. 2014, Bersani et al. 2016, Lindqvist et al. 2017, Bruenig et al. 2017). However, other questionnaires, such as the PCL-M (Devoto et al. 2016), the PCL-C (Chen et al. 2014), the SCID-I (Bersani et al. 2016; Lindqvist et al. 2017), the PDS (Himmerich et al. 2015), and the LASC (Jergović et al. 2015), were also used. Some of the studies established the diagnosis clinically based on ICD-10 criteria (Oganesyan et al. 2009) or DSM-IV (Guo et al. 2012).

Study findings: Animal Studies

All included animal studies used animal models with male Sprague-Dawley rats. Two of the studies measured TNF-α levels (Levkovitz et al. 2015, Liu et al. 2016) and one assessed TNF-α mRNA (Lee et al. 2016) in the hippocampus of stressed rats. The results of these studies are

presented in Table 1. Lee et al. (2016) induced anxiety using a single prolonged stress (SPS) procedure and found elevated TNF-α levels in the hippocampus of stressed rats, as compared to non-stressed rats. In contrast, stressed rats who received ibuprofen did not display elevated TNF-α expression; these levels did not significantly differ from non-stressed rats. In rats exposed to the predator scent stress (PSS) paradigm, Levkovitz et al. (2015) also found elevated TNF-α levels in the hippocampus, compared to rats not exposed to stress. In addition, in PSS-exposed rats treated with minocycline (a drug with anti-inflammatory capacities), normalised hippocampal TNF-α concentrations were observed. Furthermore, in both of these studies, treatment with medication resulted in a reduction in anxiety-like behaviours (as measured by the elevated plus maze test) to levels observed in non-stressed rats. Conversely, Liu et al. (2016) did not find any difference in TNF-α levels in the hippocampus of rats after SPS compared to non-stressed rats.

Table 1 here

Study findings: Human studies

Cross-sectional studies assessing TNF-α concentrations in-vivo. Nineteen cross-sectional studies assessed plasma or serum concentrations of TNF-α in patients with and without PTSD. The findings of these studies are presented in Table 2. All included studies compared concentrations of TNF-α serum or plasma levels between subjects with and without PTSD, with no studies measuring TNF-α in cerebrospinal fluid.

Serum or plasma concentrations of TNF-α were found to be significantly elevated in patients with PTSD in 12 of the included studies (von Känel et al. 2007, Hoge et al. 2009, Oganesyan et al. 2009, Vidović et al. 2011, Guo et al. 2012, Hammad et al. 2012, Mkrtchyan et al. 2013,

Chen et al. 2014, Lindqvist et al. 2014, Bersani et al. 2016, Devoto et al. 2016, Bruenig et al. 2017). Lindqvist et al. (2017) only identified this pattern at trend level. Furthermore, for one study these results became non-significant when including systolic blood pressure and time since trauma as covariates in their analyses (von Känel et al. 2007). The remaining studies found no difference in concentrations of TNF-α between those with and without PTSD (Gola et al. 2013, Zhou et al. 2014, Himmerich et al. 2015, Jergović et al. 2015, Wang et al. 2016).

Several studies also measured serum or plasma concentrations of other cytokines, including the interleukins (IL) IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, and interferon-γ (IFN-γ). Overall, results were mixed (see Table 1 for details). A high proportion of studies measuring IL-6 and IL-2 identified elevated concentrations of these cytokines in participants with PTSD, compared to those without. The majority of studies assessing concentrations of IL-1β, IFN-γ, and IL-10 reported no differences between those with and without PTSD. Investigations of IL-4 and IL-8 found contradictory results, observing both elevated and reduced concentrations of these cytokines in those with PTSD, in comparison to a control group. Taken together, these results suggest an elevation of peripherally produced TNF-α, as well as other pro-inflammatory cytokines (IL-6 and IL-2) in patients with PTSD as compared to individuals without PTSD.

Table 2 here

Cross-sectional studies investigating stimulated TNF-α production in-vitro. Five cross-sectional studies measured stimulated TNF-α production *in-vitro* comparing individuals with and without PTSD (de Kloet et al. 2007, Rohleder et al. 2007, Gill et al. 2008, Gola et al. 2013, Jergović et al. 2014). Details regarding the designs and results of these studies are presented in Table 3. In Jergović et al. (2014), the effects of PTSD on cytokine production of

phytohemagglutinin (PHA) stimulated T cells was investigated. In this study, no difference in spontaneous or stimulated TNF-α production between PTSD patients and healthy controls was detected. Gola et al. (2013) investigated spontaneous and lipopolysaccharide (LPS)-stimulated production of TNF-α in isolated PBMCs. As compared to healthy controls, refugees with PTSD spontaneously produced significantly more TNF-α. However, when including smoking as a covariate, this difference reduced to trend level only. Furthermore, no group differences were observed for stimulated TNF-α production. Rohleder et al. (2004) also found no differences between refugees with PTSD and controls in LPS-stimulated production of TNF-α. In contrast, de Kloet et al. (2007) found that PTSD patients had reduced production of LPS-stimulated TNF-α compared to healthy controls. Gill et al. (2008) measured TNF-α production in PHA and LPS-stimulated whole blood, identifying significantly higher TNF-α production in PTSD patients as compared to healthy controls and trauma controls (experienced a trauma but did not develop PTSD). This was the only study to use exclusively women in their sample. Interestingly, both Gola et al. (2013) and Gill et al. (2008) identified positive correlations between production of TNF-α (unstimulated only in Gola et al.) and PTSD symptom severity/intensity.

Table 3 here

Longitudinal studies. Three longitudinal studies investigating PTSD and TNF-α were identified (see Table 4 for further details). Two studies assessed the impact of specific interventions on TNF-α in people with PTSD (Gocan et al. 2012, Himmerich et al. 2016), with the remaining study measuring TNF-α in combat veterans with chronic PTSD who were receiving treatment-as-usual (Jergović et al. 2015). Himmerich et al. (2016) assessed German soldiers with PTSD, who were randomized to receive either inpatient psychotherapy or outpatient clinical

management. It was found that in both treatment groups, serum concentrations of TNF-α increased over the six weeks of treatment. These results remained significant when controlling for medication. In Gocan et al. (2012), soldiers with treatment-resistant PTSD were required to consume a fermented soy formulation (FSWW08) for a 3-month period. In contrast with Himmerich et al. (2016), the intervention resulted in a reduction in TNF-α plasma concentration. In Jergović et al. (2015) serum TNF-α concentration was measured at baseline and after 3 months of treatment-as-usual. No significant differences between these time points were observed. However, TNF-α could not be detected in a high percentage of participant's samples.

Table 4 here

Discussion

Summary of Findings

TNF-α in animal models of PTSD. Elevated levels of TNF-α (mRNA and protein levels) in the hippocampus of stressed rats, as compared to non-stressed rats were identified in two of the three included animal studies (Levkowitz et al. 2015, Lee et al. 2016). Furthermore, the increased levels of TNF-α were not observed in the frontal cortex or hypothalamus (Levkowitz et al. 2015). The finding of elevated levels of TNF-α production in the hippocampus of stressed rats is very interesting in light of the literature derived from human studies that reports the hippocampus to be implicated in PTSD, in terms of both volume and function (e.g. Kitayama et al. 2005, Woon et al. 2010, O'Doherty et al. 2015, van Rooij et al. 2015). Taken together, these findings suggest a potential pathophysiological role of hippocampal TNF-α production in the development of PTSD. Interestingly, when the stressed rats were administered medication with anti-inflammatory properties, the elevated TNF-α concentration reduced to the level of non-stressed rats (Levkowitz et al. 2015, Lee et al. 2016). Furthermore, this coincided with a reduction of anxiety-like behaviours in the treated rats (but not in the stressed non-treated rats) which returned to a similar level to that seen in the non-stressed rats. This highlights the therapeutic potential that immunomodulatory drugs could have for the treatment of PTSD.

TNF-α in human studies of PTSD. In accord with previous reviews and meta-analyses (Passos et al. 2015, Wang & Young 2016), concentrations of TNF-α in the plasma and serum of patients with PTSD were found to be elevated, as compared to healthy controls. Only one study demonstrated a loss of their initial statistical significance when including covariates in the analyses (von Känel et al. 2007). It is of note that in two of the remaining studies that found no difference in TNF-α concentrations between groups, TNF-α was not detectable in a high

percentage of participants (Gola et al. 2013, Jergović et al. 2015), which may account for their findings. The results of *in-vitro* investigations of TNF-α in PTSD were generally inconsistent. This is likely due to the heterogeneous methodologies used in these studies, specifically in relation to the measurement of TNF-α and the heterogeneity of the sample.

It is of interest to note that concentrations and/or production of TNF-α and other cytokines have been shown to be correlated with PTSD symptom severity (von Känel et al. 2007, Gill et al. 2008, Gola et al. 2013, Dennis et al. 2016). Specifically, concentrations of TNF-α (measured using both *in-vivo* and *in-vitro* methods) positively correlated with severity/intensity of PTSD symptoms (Gill et al. 2008, Gola et al. 2013, Bruenig et al. 2017) and severity and/or frequency of specific symptoms, including re-experiencing (Gill et al. 2008) and hyperarousal (von Känel et al. 2007, Gill et al. 2008). In von Känel et al. (2007), after including covariates in the analyses, the association only remained with hyperarousal symptoms. Given the many possible symptom combinations that are presented by individuals with PTSD (Galatzer-Levy & Bryant 2013), future research needs to consider whether inflammation is only associated with certain symptoms (O'Donovan 2016).

An increase of the production of pro-inflammatory cytokines, like TNF-α, has been found in a number of studies in which hyperproduction of TNF-α was induced by acute and chronic stress paradigms (Cosen-Binker et al. 2004, Binker et al. 2010, Liu et al. 2012, Vorhees et al. 2013). More specifically, animal studies with rats have shown that TNF-α plasma concentration increased during acute and chronic (Himmerich et al. 2013), as well as social stress (Krügel et al. 2014). However, the mechanism as to how stress leads to an increase of pro-inflammatory cytokine production is still unclear. There is evidence from the literature for several pathways by which TNF-α and other cytokines might have an effect on the brain (Quan 2008). They have

been shown to be able to activate the HPA axis, to activate neuronal serotonin transporters, to stimulate the indoleamine 2,3-dioxygenase, to contribute to the destruction of neurons, and/or to release glutamate (Zhu et al. 2006, Wichers & Maes 2002, Himmerich et al. 2009, Curran & O'Connor 2001). Therefore, stress, by inducing an increased production of pro-inflammatory cytokines, might trigger neurobiological changes, which could, as a consequence, induce psychiatric disorders such as PTSD and depression.

Taken together, it could be suggested that elevated concentrations of TNF-α could be a biomarker of PTSD. It is important to consider that TNF-α may not a specific biomarker for PTSD, but rather a general marker of psychopathology. Elevated levels of TNF-α have also been observed in other psychiatric disorders, including depression (Himmerich et al. 2008, Schmidt et al. 2014). Furthermore, given that TNF-α is part of a complex network of cytokines, which have also been shown to be elevated in PTSD and other disorders (e.g. Guo et al. 2012, Hammad et al. 2012, Schmidt et al. 2014, Bersani et al 2016), a combination of several cytokines may be the best indicator.

The data derived from longitudinal studies is difficult to interpret given the mixed findings. One study that assessed the effect of inpatient psychotherapy and clinical management found an increase in TNF-α concentration over 6 weeks of treatment (Himmerich et al. 2016). In contrast, daily consumption of a fermented soy formulation resulted in a decrease in TNF-α concentrations over the 3 month intervention (Gocan et al. 2012). However, these studies had small samples and did not include a control group. Therefore, it is unclear whether the participants with PTSD had elevated concentrations of TNF-α in comparison to healthy controls, prior to starting the intervention. Furthermore, the heterogeneity in treatment interventions means that they are not directly comparable. Interestingly, both interventions

were shown to reduce participant's scores on PTSD symptom measures. This suggests that TNF-α could potentially serve as a state marker of PTSD, given the changes in concentrations observed in response to different interventions. An additional longitudinal study found no difference in TNF-α concentrations between baseline assessment and after 3 months of treatment-as-usual (Jergović et al. 2015). However, in this study TNF-α were not detected in a high percentage of participants, so conclusions cannot be drawn. As can be seen, there is limited data on longitudinal TNF-α concentrations in treatment studies of PTSD. Therefore, future research would benefit from measuring TNF-α over the treatment course to gain a clearer understanding of how TNF-α is related to treatment response. Prospective studies would also be of benefit, given not everyone who is exposed to a traumatic event will go on to develop PTSD (Keane et al. 2009). This may be best suited to a military setting in which cytokines could be measured prior to and after deployment with additional follow-up assessments. This will help to elucidate the role of cytokines in PTSD and to determine whether elevated TNF-α is a state or trait marker of PTSD.

Methodological considerations

The findings emerging from this review must be interpreted with caution and in light of several methodological considerations. Firstly, the human studies presented here used a range of assessments to diagnose and/or measure PTSD symptomology. Assessment measures vary considerably on factors such as number of items, response format (i.e. self-report vs. interview), and the anchoring of the measure (i.e. is it anchored to a specific traumatic event, broader stressful experiences, or stressful military experience). With regards to PTSD symptom severity, this may make comparing the findings of studies difficult.

Secondly, while some studies age-matched participants and controlled for certain covariates in their analyses, the majority of the included studies did not account for pre-analytical factors that may affect the concentration of certain cytokines. These include factors such as age, BMI, smoking, medication, and concurrent diagnoses relating to physical and mental health (Dugué et al. 1994). Several studies within this review highlight the importance of this practice, finding that previously significant results became non-significant when covarying for certain factors in their analyses (e.g. systolic blood pressure: von Känel et al. 2007; smoking: Gola et al. 2013). Furthermore, recent meta-analyses have shown that TNF-α was found to be elevated in those with PTSD as compared to controls, but only when participants with comorbid depression or participants who were on medication were excluded from analyses (Passos et al. 2015, O'Donavon 2016). Thus, future studies need to carefully consider factors that may influence the measurement of cytokine concentrations and account for them within their study design and analyses.

Thirdly, the specific methodologies used to measure cytokine concentrations and production varies considerably between studies. These include using different sample types (e.g. for *in-vivo*: plasma, serum; for *in-vitro*: PBMCs, whole blood) and different equipment for measuring cytokine concentrations (e.g. ELISA, multiplex arrays). This is problematic as different methodologies may yield different results (see Zhou et al. 2015 for a review on methodological issues affecting cytokine measurement). For example, research has shown that concentrations of cytokines significantly differ between plasma and serum samples (Guo et al. 2013). As a result, the findings from these studies may not be directly comparable.

Finally, the majority of the samples in the included studies have small samples and participants are limited to males and to those suffering from PTSD due to war i.e. veterans and refugees.

Therefore, we cannot be sure that the presented findings will apply to those experiencing PTSD due to being exposed to a different trauma e.g. accident, natural disasters, terrorist attacks (Wang & Young 2016). Also, some studies used a trauma control group (i.e. they were exposed to trauma but did not go on to develop PTSD; e.g. von Känel et al. 2007, Bruenig et al. 2017) as opposed to healthy control group (i.e. no experience of trauma). At this point, it is unclear as to what extent trauma exposure can influence inflammatory markers in the long-term, even without the development of PTSD (Passos et al. 2015).

Conclusions

To our knowledge, this is the first systematic review considering the specific role of TNF-α in PTSD. The current review indicates that (i) generally serum and plasma concentrations of TNF-α are elevated in those with PTSD in comparison to those without; thus suggesting that TNF-α may be a potential biomarker of PTSD and serve as a potential therapeutic target for PTSD (Neigh & Ali 2016); (ii) TNF-α production in the hippocampus may be involved in the underlying pathophysiology of PTSD; and (iii) in animal models of PTSD, anxiety-like behaviour can be altered by immunomodulatory drugs, which highlights the future potential of this medication for the treatment of PTSD. However, these findings do need to be interpreted in view of methodological issues and the potential for publication bias (Thornton & Lee 2000). Longitudinal research is needed to understand the state/trait related nature of TNF-α concentrations in PTSD. This will enlighten us to the potential biological mechanisms underlying PTSD, which may be responsible for the development and/or maintenance of the disorder, elucidate if cytokines concentrations could be a potential marker of treatment response, and may provide the basis for further investigations into immunomodulatory medication as a treatment for PTSD.

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Table 1. Summary of animal studies investigating the relationship between TNF-α and PTSD.

Author	Animals	PTSD Model	Measurement of TNF-α	Findings regarding TNF-α	Additional findings/comments
Lee et al. 2016	Adult male Sprague-Dawley rats	SPS	Hippocampal mRNA expression levels; Reverse transcription-polymerase chain reaction	SPS > control	Group treated with ibuprofen (40mg/kg body weight): TNF-α - SPS + ibuprofen = control; SPS + ibuprofen < SPS Anxiety index in elevated plus maze test - SPS > SPS + ibuprofen; SPS + ibuprofen = control
Levkowitz et al. 2015	Male Sprague-Dawley rats (n=99)	PSS	Hippocampal protein levels; Multiplexed ELISA	PSS > control	In frontal cortex & hypothalamus: TNF-α - PSS = control Group treated with minocycline: TNF-α - PSS > PSS + minocycline

					IL-1α, IL-6 - PSS > control; PSS > PSS + minocycline Anxiety-like behaviours (time spent in open arms and entries) in elevated plus maze test - PSS > control; PSS > PSS + minocycline; PSS + minocycline = control
Liu et al. 2016	Adult male Sprague-Dawley rats (n=128)	SPS	Hippocampal expressions; ELISA	SPS = control	IL-6 - SPS > control IL-1β, IL-10 - SPS = control

TNF-α = tumor necrosis factor - alpha; SPS = single prolonged stress; mRNA = messenger ribonucleic acid; PSS = predator scent stress; ELISA = enzyme-linked immunosorbent assays; mg = milligram; kg = kilogram; IL = interleukin

Table 2. Human cross-sectional studies assessing plasma or serum concentrations of TNF-α *in-vivo* in individuals with and without PTSD.

Author	N	Sample	PTSD Assessment	Measurement of TNF-α	Findings regarding TNF-α	Additional findings/comments
Bersani et al. 2016	111	Male combat veterans with (n=56; n=28 concurrent MDD) and without (n=55; HC) current PTSD	CAPS and SCID	Serum; High sensitivity multiplexed sandwich immunoassay	PTSD > HC	IL-6 - PTSD > HC IL-1β, IFN-γ - PTSD = HC Total pro-inflammatory score (TNF-α, IL-6, IL-1β, IFN-γ & CRP) - PTSD > HC NB includes participants from Lindqvist et al. (2014).
Bruenig et al. 2017	299	Male trauma-exposed Vietnam War veterans with (n=159) and without PTSD (n=140)	CAPS-5	Serum; Luminex 100 Milliplex cytokine multiplex bead assay	PTSD > trauma control In the whole sample (but not in PTSD group only), TNF-α correlated positively	Groups matched for age.

					with PTSD symptom severity.	
Chen et al. 2014	120	Individuals of Li ethnicity with PTSD (n=30), individuals of Han ethnicity with PTSD (n=30), HC of Li ethnicity (n=30), and HC of Han ethnicity (n=30)	PCL-C PTSD according to DSM-IV	Serum; ELISA	For both ethnicities: PTSD > HC	For both ethnicities: IL-2, IL-6, IL-8 - PTSD > HC
Devoto et al. 2016	63	Active duty personnel with a history of traumatic brain injury (TBI) with low (n=35) and high (n=28) PTSD	PCL-M	Plasma; Paramagnetic bead-based ELISA	High PTSD > Low PTSD	IL-6 - High PTSD > Low PTSD IL-10 - High PTSD = Low PTSD IL-6 & TNF-α - TBI > no history of TBI
Gola et al. 2013	60	Refugees with PTSD (n=35; n=27	CAPS	Plasma; Multiplex bead-based assays	PTSD = HC	Results remained true when including sex or smoking as a covariate.

		concurrent MDD) and HC (n=25)	PTSD diagnosis according to DSM-IV		74.6% of samples were below detection limit for TNF-α No difference also observed in second assessment at one week after baseline.	IL-6, IL-10 - PTSD = HC IL-8 - PTSD < HC Groups matched for ethnicity. See also Table 3 for *in-vitro* cross-sectional data.
Guo et al. 2012	100	Individuals with and (n=50; n=22 males) without (n=50; n=25 males; HC) PTSD	DSM-IV	Serum; ELISA	PTSD > HC	IL-2, IL-4, IL-6, IL-8, IL-10 - PTSD > HC Groups matched for age and gender.
Hammad et al. 2012	13	Combat veterans with PTSD (n=8; n=6 concurrent MDD) and male HC (n=5)	CAPS-DX	Plasma; Human cytokine 4-plex panel	PTSD > HC	IL-6, IL-10, IFN-γ - PTSD > HC

Himmerich et al. 2015	135	Male German soldiers	PDS	Serum; BioPlex ProTM human cytokine immunoassay	PTSD = no PTSD No significant correlation between PDS score and TNF-α concentrations.	sTNF-R p55 & p75 - PTSD = no PTSD Controlled for smoking, BMI and age.
Hoge et al. 2009	56	Individuals with PTSD (n=28) and HC (n=28)	SPRINT PTSD diagnosis according to DSM-IV	Plasma; Millipore Beadlytes Human 22-Plex Multi-Cytokine Detection System and the Luminex 100 Total System	PTSD > HC	IL-6, IL-1a, IL-1b, IL-2, IL-4, IL- 7, IL-8, IL-10, IL12p40 and IL12p70, IL-13, IL-15, IP-10 - PTSD > HC Groups matched for gender and age.
Jergović et al. 2015	101	Male combat veterans with PTSD (n=69) and male HC (n=32)	LASC PTSD diagnosis according to ICD-10	Serum; Bead-based multiplex immunoassay	PTSD = HC TNF-α not detected in 80% of PTSD and 87% of HC samples	IL-8 - PTSD < HC IFN-γ, IL-1β, IL-2, Il-4, IL-6 - PTSD = HC Groups matched for age.

						See also Table 4 for longitudinal data.
Lindqvist et al. 2014	102	Male combat-exposed veterans with PTSD (n=51; n=27 concurrent MDD) and male combat-exposed HC (n=51)	CAPS PTSD diagnosis according to DSM-IV	Serum; High sensitivity multiplexed sandwich immunoassay	PTSD > HC	IL-1β, IL-6, IL-10 - PTSD = HC IFN-γ - PTSD > HC Controlled for MDD diagnosis, BMI, BDI score, ETI score, use of antidepressants, anti-inflammatories and statins, asthma/allergy illnesses, ethnicity, years of education, and time since combat. Groups matched for age.
Lindqvist et al. 2017	61	Male combat-exposed veterans with PTSD (n=31;	CAPS	Serum; High sensitivity	PTSD > HC (trend only)	Total pro-inflammatory score, IL-6 - PTSD > HC

		n=20 concurrent MDD) and male combat-exposed HC (n=30)	PTSD diagnosis according to DSM-IV	multiplexed sandwich immunoassay		IFN- γ, IL-10 - PTSD = HC Controlled for age, BMI, smoking, medications and immune/ inflammatory illnesses. Total pro-inflammatory score - PTSD + MDD = PTSD no MDD NB Replication of Lindqvist et al. (2014)
Mkrtchyan et al. 2013	72	Male war veterans with PTSD (n=37) and male HC (n=35)	SCDI-I and CAPS PTSD diagnosis according to DSM-IV-TR	Serum; ELISA	PTSD > HC	

Oganesyan et al. 2009	62	Individuals with chronic stage PTSD (n=31, n=27 male) and HC (n=31, n=27 male)	ICD-10	Serum; ELISA	PTSD > HC	IL-1β, IL-6 - PTSD > HC
Vidović et al. 2011	64	Male Croatian combat veterans (n=39) and male HC (n=25)	CAPS PTSD diagnosis according to ICD-10	Serum; ELISA	PTSD > HC Results collected at second assessment (average 5.6 years after baseline): PTSD = HC	IL-6 - PTSD = HC
von Känel et al. 2007	28	Individuals with PTSD (n=14; n=9 male) and trauma control (n=14; n=9 male)	CAPS (German Version) PTSD diagnosis according to DSM-IV	Plasma; Ultra-sensitive enzyme-linked immunosorbent assay	PTSD > trauma control TNF-α correlated negatively with systolic BP, and positively with time since trauma. TNF-α	Results become insignificant when controlling for systolic BP and time since trauma. IL-1β (controlling for anxiety & depression),

					positively correlated with re-experiencing, avoidance, hyperarousal, and total PTSD symptom score, association became non-significant when adjusting for systolic BP and time since trauma (for all except hyperarousal).	IL-6, IL-10 - PTSD = trauma control IL-4 (controlling for systolic BP & smoking status) - PTSD < trauma control Groups matched for gender and age. Trauma control: All had experienced traumatic accident but did not develop PTSD.
Wang et al. 2016	13	OEF/OIF Veterans with (n=7; n=4 concurrent MDD) and with without (n=6) PTSD	CAPS	Plasma; Human cytometric bead array flex sets	PTSD = no PTSD	IL-2, IFN-γ, IL-6, IL-17 - PTSD > no PTSD IL-4 - PTSD < no PTSD IL-10 - PTSD = no PTSD

Zhou et al. 2014	72	Combat veterans with PTSD (n=30, n=27 male) and HC (n=42)	CAPS and PCL-M	Plasma; Bio-Plex Luminex 100 system	PTSD = HC	IFN-γ, IL-17 - PTSD > HC IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15 - PTSD = HC Groups matched for age.

TNF-α = tumor necrosis factor - alpha; PTSD = post-traumatic stress disorder; ELISA = enzyme-linked immunosorbent assays; IL = interleukin; IFN = interferon; MDD = major depressive disorder; OEF/OIF = Operation Enduring Freedom/Operation Iraqi Freedom; HC = healthy control; CAPS = Clinician- Administered PTSD scale; PCL-C = PTSD Checklist - Civilian Version; PCL-M = PTSD Checklist - Military Version; SCID-I = Structured Clinical Interview for DSM Disorders; LASC = Los Angeles Symptom Checklist; SPRINT = Short Post-Traumatic Stress Disorder Rating Interview; PDS = Posttraumatic Diagnostic Scale; BP = blood pressure; CRP = C-reactive protein; BDI = Beck Depression Inventory; ETI = Early Trauma Inventory; BMI = body mass index.

Table 3. Human cross-sectional studies assessing TNF-α production *in-vitro* in individuals with and without PTSD.

Author	N	Sample	PTSD Assessment	Measurement of TNF-α	Findings regarding TNF-α	Additional findings/comments
de Kloet et al. 2007	83	Male veterans with PTSD (n=29; n=14 concurrent MDD), male trauma control (n=29; veterans without PTSD) and male HC (n=25)	CAPS	Whole blood; NR Stimuli: LPS	PTSD < HC PTSD = trauma control Trauma control = HC PTSD with MDD = PTSD without MDD	IL-10 - PTSD = HC = trauma control Groups matched on age, region and year of deployment.
Gill et al. 2008	76	Females with PTSD (n=26; n=13 concurrent MDD), female trauma controls (n=29; past trauma but no PTSD), and female	CAPS	Whole blood; ELISA Stimuli: PHA plus LPS	PTSD > trauma control PTSD > HC Trauma control = HC	IL-6 - PTSD > trauma control; PTSD > HC IL-1β - PTSD = HC = trauma control Controlled for age, BMI and smoking.

		HC (n=21; no past trauma and no PTSD)			PTSD with MDD > PTSD without MDD (trend only) TNF-α positively correlated with PTSD symptom intensity, re-experiencing symptoms, hyperarousal symptoms, & intensity of depression.	
Gola et al. 2013	34	Refugees with PTSD (n=16) and HC (n=18) In total sample, see Table 2: N=27 PTSD	CAPS PTSD diagnosis according to DSM-IV	PBMCs; Multiplex bead-based assays Stimuli: LPS or unstimulated	Unstimulated: PTSD > HC LPS-stimulated: PTSD = HC	Unstimulated TNF-α results reduced to trend level when smoking included as a covariate. LPS-stimulated results remained true when

		patients met DSM-IV criteria for MDD			Unstimulated TNF-α production positively correlated with PTSD symptom severity.	including sex or smoking as a covariate. Unstimulated: IL-1β, IL-6 - PTSD > HC LPS-stimulated: IL-1β - PTSD = HC; IL-6 - PTSD > HC Groups matched on ethnicity. See also Table 2 for *in-vivo* cross-sectional data
Jergović et al. 2014	101	Male combat veterans with PTSD (n=30; n=24 concurrent MDD) and male HC (n=17)	CAPS PTSD diagnosis according to ICD-10	PBMCs; NR Stimuli: LPS or unstimulated	PTSD = HC	Stimulated: IFN-γ, IL-2, IL-4 - PTSD=HC Unstimulated: IL-4 - PTSD = HC; IFN-γ, IL-2 - PTSD < HC

						Telomere length - PTSD < HC
Rohleder et al. 2004	25	Bosnian refugees with PTSD (n=12, n=7 males; n=2 concurrent MDD) and HC (n=13, n=5 males)	SCL-90R and HTQ PTSD diagnosis according to DSM-IV	Whole blood; ELISA Stimuli: LPS	PTSD = HC	Groups matched on age. IL-6 - PTSD > HC Groups matched for age and gender.

TNF-α = tumor necrosis factor - alpha; PTSD = post-traumatic stress disorder; ELISA = enzyme-linked immunosorbent assays; IL = interleukin; IFN = interferon; MDD = major depressive disorder; HC = healthy control; NR = not reported; CAPS = Clinician- Administered PTSD scale; BMI = body mass index; HTQ = Harvard Trauma Questionnaire; SCL-90R = Symptom Checkist-90-Revised; PBMC = peripheral blood mononuclear cell; LPS = lipopolysaccharide; PHA = phytohaemagglutinin.

Table 4. Human longitudinal studies assessing TNF-α concentration in individuals with PTSD.

Author	N	Sample	Intervention	PTSD Assessment	Measurement of TNF-α	Findings regarding TNF-α	Additional findings/comments
Gocan et al. 2012	10	Male treatment-resistant soldiers with combat-related PTSD	Consumption of a fermented soy formulation: FSWW08 (120ml daily)	CAPS PTSD diagnosis according to ICD-10	Plasma; ELISA At baseline (T0) and after intervention (3 months; T1)	T0 > T1	IL-1β, IFN-γ - T0 > T1 IL-6 - T0 = T1 CAPS score - T0 > T1
Himmerich et al. 2016	38	Male German soldiers with PTSD NB recruited from Himmerich et al. (2015, 2016) - See Table 1	Randomised to receive immediate inpatient psychotherapy (n=21) or outpatient clinical management control group (n=17)	PDS	Serum; Bio-Plex Pro™ human cytokine immunoassay At baseline (T0) and after treatment (six weeks; T1)	T0 < T1 No correlations between change in PDS score and change in TNF-α	sTNF-R p55, sTNF-R p75 - T0 > T1 PDS score - T0 > T1 Results remained significant when controlling for medication.
Jergović et al. 2015	69	Male Croatian combat veterans with PTSD	N/A TAU	LASC PTSD diagnosis according to ICD-10	Serum; Bead-based multiplex immunoassay	T0 = T1 TNF-α not detected in high percentage of participants	See Table 2 for *in-vivo* cross-sectional data.

	At baseline (T0) and after 3 months (T1)

TNF-α = tumor necrosis factor - alpha; PTSD = post-traumatic stress disorder; ELISA = enzyme-linked immunosorbent assays; IL = interleukin; IFN = interferon; CAPS = Clinician- Administered PTSD scale; LASC = Los Angeles Symptom Checklist; PDS = Posttraumatic Diagnostic Scale; sTNF-R = soluble tumor necrosis receptor; T0 = baseline time point; T1 = first time point; N/A = not applicable; TAU = treatment-as-usual.