RESEARCH ARTICLE

Serum concentrations of TNF-α, sTNF-R p55 and p75 and post-traumatic stress in German soldiers

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ABSTRACT. Growing evidence suggests involvement of the tumor necrosis factor (TNF)-α system in the pathophysiology of psychiatric disorders. Research into post-traumatic stress disorder (PTSD) has investigated serum levels of TNF-α, but not to date its soluble receptors sTNF-R p55 and sTNF-R p75. We examined serum levels of TNF-α, sTNF-R p55 and sTNF-R p75 in 135 male German soldiers 70 of whom had been deployed abroad and 65 in Germany only. Post-traumatic stress symptoms were measured using the Post-traumatic Stress Diagnostic Scale (PDS) and the Trier Inventory for the Assessment of Chronic Stress (TICS). Correlational analysis controlling for multiple testing, showed no significant Spearman rank correlations between PDS or TICS scores and serum levels of TNF-α, sTNF-R p55 or sTNF-R p75, either in the full sample or in the group of soldiers who had been deployed abroad. ANCOVAs showed no significant differences between soldiers with or without a PDS-derived diagnosis of PTSD, or between soldiers with or without deployment abroad, after controlling for age, smoking and body mass index (BMI). These results suggest that the TNF-α system, as reflected by TNF-α, sTNF-R p55 and sTNF-R p75 serum levels, does not play a major role in the pathophysiology and development of PTSD symptoms as measured by the PDS and the TICS. However, several methodological and contextual issues have to be considered.

Key words: cytokines, tumor necrosis factor (TNF)-alpha, TNF receptors, post-traumatic stress disorder

In recent years, growing evidence has pointed to the involvement of cytokines, and more specifically tumor necrosis factor (TNF)-α, in the pathophysiology of neurological and psychiatric disorders such as multiple sclerosis, Parkinson's and Alzheimer's disease, depression, schizophrenia and narcolepsy [1]. Elevated levels of TNF-α and its soluble receptors sTNF-R p55 and p75 have been repeatedly found in depressed patients in particular [2, 3]. The direct and indirect effects of TNF-α on neurotransmitter storage and release have been discussed as pathophysiological factors in depression [4], and anti-TNF-α medication has been proposed as an antidepressant [5, 6].

Along with depression, post-traumatic stress disorder (PTSD) has been conceptualized as a stress-related disorder [7]. In animal models of stress, different kinds of acute or chronic stress [8-10] lead to elevated TNF-α levels in both blood and brain. In human stress paradigms, stress consistently up-regulates TNF-α production [11, 12]. Serum levels of TNF-α, sTNF-R p55 and sTNF-R p75 have been found to be elevated in depressed patients [13]. In PTSD, TNF-α levels have been found to be associated with post-traumatic stress in some [14, 15], but not all studies [16]. To our knowledge, the levels of sTNF-R p55 and sTNF-R p75 in the serum of PTSD patients have not been reported.

PTSD can be diagnosed categorically, but there are also standardized interviews available to quantify post-traumatic stress symptomatology. The Post-traumatic Stress Diagnostic Scale (PDS) was developed and validated by Foa [17] to provide a brief but reliable, self-reported measure of PTSD for use in both clinical and research settings [17-19]. In order to obtain information about the influence of overall chronic stress on the TNF-α system in these subjects, we also applied the Trier Inventory for the Assessment of Chronic Stress (TICS) developed by Schulz, Schlotz, and Becker, which captures chronic psychosocial stress [20, 21].

In a sample of male German soldiers, we investigated the relationship between post-traumatic stress, as measured by the PDS, and parameters of the TNF-α system, namely TNF-α, as well as its receptors sTNF-R p55 and sTNF-R p75. To our knowledge, this is the first report examining a potential association between post-traumatic stress

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as measured by the PDS, and the TNF-α system. Up to now, only interleukin (IL)-4, IL-6, IL-8 and transforming growth factor (TGF)-ß serum levels have been analyzed in the context of post-traumatic stress as measured by the PDS [22].

METHODS

Subjects

One hundred and thirty five male German soldiers of mean age 30.3 (SD 6.8) years participated. Exclusion criteria were female sex and current psychotherapy for PTSD. Of the 135 soldiers, 70 had served in deployments abroad, 65 had only served within Germany to date; sixty-five smoked tobacco daily, 48 were non-smokers, and 22 did not provide information regarding their smoking habits. The mean body mass index (BMI) of the participants was 26.00 kg/m^2, and ranged from 19.41 to 36.44 kg/m^2. Regarding the medical history of the study participants, the main previous physical health problems were back (N = 3), knee (N = 2), teeth (N = 2) and intestinal problems (N = 2). The study began in December 2011, ended in June 2014, and included clinical and self-reported interviews, physical examination, and standardized blood sampling performed in the morning.

Psychological measures

Post-traumatic Stress Diagnostic Scale (PDS) (German version).

The PDS was developed and validated by Foa [17] to provide a brief, but reliable, self-reported measure of PTSD for use in both clinical and research settings. The test is self-administered, can usually be completed in 10-15 minutes, and requires a reading age of around 13 years. Test items mirror the DSM-IV criteria for PTSD. Questions relate to the frequency of distressing and intrusive thoughts, post-traumatic avoidance, and hyperarousal [17, 18]. The PDS includes a symptom severity score which ranges from 0 to 51. In accordance with recommendations in the literature, cut-offs used for symptom severity rating were: 0-10 mild (i.e. not clinically significant), 11-20 moderate, 21-35 moderate to severe and ≥36 for severe PTSD symptomatology [17, 18]. The PDS has very good reliability and validity, with internal consistency ranging from α = 0.78 to α = 0.98 for the overall score [19]. In the present study, a PTDS score of 11 or more was taken to represent a diagnosis of PTSD and a PDS score of 10 or less, no diagnosis of PTSD.

Trier Inventory for the Assessment of Chronic Stress (TICS).

The TICS was developed by Schulz, Schlotz, and Becker, and captures chronic psychosocial stress [20, 21]. It rates 57 items on a five-point Likert scale to show how often subjects have experienced specified situations or have had specified experiences within the previous three months. Studies have shown very good reliability and validity for the TICS, with internal consistency ranging from α = 0.84 to α = 0.91 [20, 21].

Cytokine measurements

After blood sampling, serum probes were immediately centrifuged at 3000 rpm for 10 min. The supernatant was aliquoted and stored in 300 µL, non-absorbing, polypropylene tubes. Probes were shock-frozen in liquid nitrogen and stored in freezers at -80°C until further measurement. TNF-α and its soluble receptors sTNF-R p55 and p75 were measured in the serum using the Bio-Plex Pro™ human cytokine immunoassay from Bio Rad, Germany.

Statistics

All statistical tests were performed using SPSS 21. We corrected for multiple testing using Bonferroni's method. Based on an initial level of significance of p = 0.05, two different questionnaires (PDS and TICS), and three different laboratory parameters (TNF-α, sTNF-R p55 and sTNF-R p75), the corrected level of significance was set at p* = 0.008.

Ethics

The study was approved by the local ethics committee (Ethikausschuss Charité, application number: EA1/270/11).

RESULTS

Descriptive statistics

In the full sample (N = 135), PTSD symptom severity ratings on the PDS were: 88 subjects (65.2%) not clinically significant, 10 subjects (7.4%) moderate, 19 subjects (14.1%) moderate to severe, and 18 subjects (13.3%) severe. The mean (SD) PDS score was 13.07 (16.28) and TICS score 13.90 (10.50).

TNF-α serum levels ranged from 0.73 to 1,181.82 pg/mL, median 4.95 pg/mL. sTNF-R p55 concentrations ranged from 130.68 to 824.15 pg/mL, median 342.57 pg/mL. sTNF-R p75 levels ranged from 410.83 to 2620.21 pg/mL, median1122.90 pg/mL.

Relationship between stress and the TNF-α system

After controlling for multiple testing, the Spearman rank correlation analyses did not reveal any significant correlation between the PDS and TICS scores and serum levels of TNF-α, sTNF-R p55 or sTNF-R p75, in either the full sample or the group of soldiers who had been deployed abroad. For details regarding the correlation coefficients and the 2-tailed level of significance see *table 1*.

ANCOVAs showed no significant differences in laboratory measures between soldiers with or without a PDS-derived diagnosis of PTSD, or between soldiers with or without deployment abroad, after controlling for age, daily tobacco use and BMI. For example, ANCOVA for sTNF-R p55 serum levels revealed no significant group difference between soldiers with and without PTSD ($F[1, 100] < 1$; n.s.), with BMI showing the highest impact ($F[1,100] = 7.36$; $p = .008$) on sTNF-R p55 levels when testing for inter-subject effects. Detailed statistical information is presented for this example, because given the

Table 1
Spearman rank correlations (r: correlation coefficient; p: 2-tailed level of significance) between TNF-α, sTNF-R p55 and sTNF-R p75 serum levels [pg/mL] and PDS and TICS scores. None of the tested correlations was significant according to the corrected level of significance of p* = .008.

		Whole group (N = 135)		Soldiers deployed abroad (N = 70)		Soldiers deployed in Germany (N = 65)	
		PDS	TICS	PDS	TICS	PDS	TICS
TNF-α	r	.000	-.078	-.064	-.009	.051	-.163
	p	.998	.394	.619	.943	.701	.219
sTNF-R p55	r	.224	.195	.264	.241	-.002	.054
	p	.010	.026	.030	.048	.989	.673
sTNF-R p75	r	.167	.101	.087	.097	.126	.058
	p	.060	.255	.492	.447	.321	.647

rank correlation coefficients (see *table 1*), we expected the strongest association to be between sTNF-R p55 levels and the PTSD questionnaire scores.

DISCUSSION

The main finding of the present study is that there were no significant correlations between the PTDS or TICS scores and the serum levels of TNF-α, sTNF-R p55 or sTNF-R p75. This negative result was found in the full sample and in the group of soldiers who had been deployed abroad. This finding is in contrast to some [14, 15], but not all [16] previous studies.

Several issues have to be taken into account when interpreting this negative result: this was the first study investigating PTSD and stress symptoms using the PDS and the TICS. As the assessments and criteria for a PTSD diagnosis differed from those of former studies, our results are not fully comparable. To our knowledge, we investigated the largest sample of soldiers to date. Therefore, smaller studies may have overestimated the association between PTSD symptoms and the TNF-α system. Moreover, the majority of previous positive studies did not correct for multiple testing as we did. With respect to comparability, one consideration is that the present study included only male soldiers, whereas a number of other studies included females as well as males (for example [14]).

A further important issue is the meaning of the non-significant associations between the PDS score and TNF receptor levels after controlling for BMI. The negative finding could reflect that these receptors do not actually play a major role in the pathophysiology of PTSD. However, due to the limited sample size we cannot rule out that the correlations between parameters of the TNF-α system and PTSD symptoms may become statistically significant in larger samples. It is also noted that our study design is cross-sectional and retrospective. Hence, for example, soldiers with PTSD symptoms might have had lower values of the parameters of the TNF-α system before they experienced threatening situations during their deployment abroad. Whether the parameters investigated are or are not biomarkers of PTSD should be decided only on the basis of longitudinal studies.

Some previous studies (such as [15]) took the BMI into account as a covariate; others however, (such as [14]) did not. Our data suggest that BMI is a crucial variable, influencing sTNF-R p75 serum levels. It is also known from prior research, that the BMI is associated with TNF receptor p55 and 75 serum levels [23], and may therefore be a major factor for the production of TNF receptor levels. Therefore, BMI should be included in further investigations into the association of PTSD with parameters of the TNF-α system.

In conclusion, we investigated quantitatively-measured, post-traumatic stress symptoms and TNF-α, sTNF-R p55 and p75 serum concentrations. We found no significant association between PTSD symptoms and parameters of the TNF-α system. However, further research is needed before final conclusions can be drawn. This would include longitudinal studies measuring TNF-α system levels before and after trauma, and TNF-α system responses during the course of specific psychological and pharmacological therapies.

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