Cytokine production as a putative biological mechanism underlying stress sensitization in high combat exposed soldiers

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KEYWORDS
Posttraumatic stress disorder; Stress sensitization; Military; Cytokines; Chemokines

Summary
Objective: Combat stress exposed soldiers may respond to post-deployment stressful life events (SLE) with increases in symptoms of posttraumatic stress disorder (PTSD), consistent with a model of stress sensitization. Several lines of research point to sensitization as a model to describe the relations between exposure to traumatic events, subsequent SLE, and symptoms of PTSD. Based on previous findings we hypothesized that immune activation, measured as a high in vitro capacity of leukocytes to produce cytokines upon stimulation, underlies stress sensitization.

Methods: We assessed mitogen-induced cytokine production at 1 month, SLE at 1 year, and PTSD symptoms from 1 month up to 2 years post-deployment in soldiers returned from deployment to Afghanistan (N = 693). Exploratory structural equation modeling as well as latent growth models were applied.

Results: The data demonstrated significant three-way interaction effects of combat stress exposure, cytokine production, and post-deployment SLE on linear change in PTSD symptoms over the first 2 years following return from deployment. In soldiers reporting high combat stress

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1. Introduction

Exposure to traumatic stressors may increase an individual’s reactivity to subsequent stressors, a process that has been termed stress sensitization (Antelman et al., 1980; Post and Weiss, 1998). Stress sensitization has been proposed by several authors to play a role in the development of symptoms of posttraumatic stress disorder (PTSD) after exposure to traumatic events (Antelman and Yehuda, 1994; McFarlane, 2010). Consistent with the stress sensitization hypothesis, prior exposure to traumatic life events has been found to sensitize a person to the impact of subsequent stressors (Bland et al., 1996; Breslau et al., 1999; Dougall et al., 2000; Kessler et al., 1995; King et al., 1996; Smith et al., 2008). A number of prospective studies (Grasso et al., 2012; Smid et al., 2012, 2013) have provided evidence for stress sensitization following exposure to traumatic events. In a study of Dutch soldiers (Smid et al., 2013), high combat stress exposure was found to be associated with sensitization to the effects of post-deployment stressors during the first year following return from deployment. Specifically, a steeper linear increase in PTSD symptoms post-deployment was predicted by more post-deployment stressors in high combat stress exposed soldiers, but not in a less combat stress exposed group. A protective effect of exposure to potentially traumatic events on subsequent stress reactivity, often termed stress inoculation, has been found in persons who were only mildly affected and who managed to cope successfully (Norris and Murrell, 1988; Parker et al., 2004; Seery et al., 2010). Sensitization has also been described in conjunction with kindling in PTSD (Post and Weiss, 1998). Sensitization refers to externally induced symptoms, e.g., flashbacks of traumatic events induced by a subsequent stressor, whereas kindling refers to spontaneous symptoms, e.g., flashbacks occurring in the absence of an apparent cue. It has been suggested that kindling may follow sensitization, e.g., when flashbacks are triggered by progressively less severe stressors over time and eventually occur spontaneously (Post and Weiss, 1998).

Exposure to potentially traumatic events as well as symptoms of PTSD have been linked with alterations in the functioning of the immune system (Page and Heim, 2011). Indeed, an accumulating body of evidence suggests that cytokines subserve processes such as learning and memory, that are involved in the pathogenesis of PTSD (Baker et al., 2012). A number of studies have investigated the capacity of peripheral leukocytes of individuals with PTSD symptoms to produce cytokines after in vitro stimulation (de Kloet et al., 2007; Gill et al., 2008; Gola et al., 2013; Kawamura et al., 2001; Rohleder et al., 2004; Woods et al., 2005). Increased (Gill et al., 2008; Rohleder et al., 2004; Woods et al., 2005), unchanged (Gola et al., 2013), and decreased (de Kloet et al., 2007) mitogen-induced pro-inflammatory cytokine production in individuals with PTSD symptoms compared to non-traumatized controls has been found, as well as decreased production in individuals with a past history of PTSD (Kawamura et al., 2001). Increased non-stimulated production of pro-inflammatory cytokines in PTSD patients compared with controls has also been reported (Gola et al., 2013). Thus, the predominant finding amongst three studies appears to be that cytokine production is increased in PTSD as compared to non-traumatized controls. The discrepancy in study findings may be explained by differences in sample characteristics, such as the type of trauma experienced (e.g., childhood vs. adult trauma), time elapsed since the trauma, comorbid disorders, PTSD symptom severity, and possibly exposure to SLE after the traumatic event.

In a previous study from our group (Van Zuiden et al., 2011b), high stimulated pre-deployment T-cell cytokine production predicted development of depressive symptoms in response to military deployment, as measured 6 months after return. This study used exploratory structural equation modeling (ESEM), an advanced statistical technique, to diminish redundancy in cytokine data stemming from functional overlap. Using this exploratory technique, functionally distinct cytokine/chemokine factors originating from the innate and adaptive immune system were identified, specifically, T-cell cytokines, T-cell chemokines (including IL-6), and innate cytokines.

This same study (Van Zuiden et al., 2011b) reported an increase in stimulated T-cell cytokine production from pre-deployment to 6 months post-deployment, indicating that deployment to a combat-zone increases the capacity of T-cells to produce cytokines until at least 6 months after return. Conversely, stimulated T-cell chemokine/IL-6 production as well as innate cytokine production after stimulation with Lipopolysaccharide decreased during this interval. Extrapolating from these results, it may be hypothesized that this increased T-cell cytokine production capacity in response to deployment could confer an increased risk for development of depressive symptoms after confrontation with new stressors (Van Zuiden et al., 2011b). Thus, increased T-cell cytokine production may be involved in the previously observed stress sensitization in high combat stress exposed soldiers. This is in line with previously reported evidence (Hayley et al., 2003) that the pro-inflammatory cytokine TNF-α sensitizes neural systems.
Cytokine production underlying stress sensitization

2. Methods

2.1. Sample

This study is part of a prospective longitudinal cohort study in Dutch military personnel. From 2005 to 2010, the Netherlands Armed Forces deployed approx. 20.000 soldiers to Afghanistan as part of an International Security Assistance Force (ISAF). Participants in this study volunteered to participate prior to a 4-month deployment to Afghanistan between 2005 and 2008. Duties during deployment of this group consisted of combat patrols, clearing or searching buildings, participation in de-mining operations, and transportation across enemy territory. Combat experiences included exposure to enemy fire, participation in armed combat, seeing seriously injured comrades and civilians, and witnessing the death of fellow soldiers and civilians. The study comprised 5 assessments: approximately 2 months prior to deployment (T0) and approximately 1 (T1), 6 (T2), 12 (T3), and 24 (T4) months following return from deployment. The study was approved by the Institutional Review Board of the University Medical Center Utrecht, the Netherlands. Written informed consent was obtained after a written and verbal description of the study. The first three assessments took place at military bases or in the military hospital and also involved blood sampling; the last two assessments were mailed or provided online. Data from the sample have been described in several publications from our group (e.g., Van Zuiden et al., 2011b). The present study is based on a subsample that included all participants belonging to the so-called Task Force Uruzgan (TFU) rotations, who were administered a stressful life-events checklist one year after deployment (Smid et al., 2013).

Fig. 1 shows a flow chart of study participants, providing details on the number of participants who missed an assessment or dropped out from the study. At T0, N = 814 constituted the initial sample. The number of participants at T1 was N = 693 (85.1% of the initial sample); at T2: N = 644 (79.1%); at T3: N = 465 (57.1%); and at T4: N = 433 (53.2%). Of the 814 participants at T0, N = 345 (42.4%) participated in all 5 assessments; N = 146 (17.9%) participated in 4, N = 167 (20.5%) in 3, N = 82 (10.1%) in 2, and N = 74 (9.1%) in 1 assessment. Study participants who dropped out from one or more study assessments differed from those who completed all assessments in the following baseline characteristics: younger age (24.6 vs. 30.4, p < .001), lower rank (soldier or corporal: 80.8% vs. 47.4%, p < .001), lower education (low educational level: 51.0% vs. 30.7%, p < .001), lower Body Mass Index (24.3 vs. 24.9, p < .009), and fewer previous deployments (0.61 vs. 1.09, p < .001). In addition, study attrition was associated with more deployment stressors (4.32 vs. 3.68, p = .002), and higher PTSD symptoms at T2 (28.94 vs. 27.47, p < .017). Study participants who dropped out from one or more study assessments did not differ from those who completed all assessments in gender, early life trauma, post-deployment stressors, and PTSD symptoms at T1, T3 and T4. Because demographic variables (age, thereby increasing vulnerability to the depressive effects of stressors.

In the aforementioned study (Van Zuiden et al., 2011b), cytokine production was not associated with the severity of PTSD symptoms 6 months following deployment or the number of retrospectively reported deployment stressors. However, the interaction between cytokine production and combat stress exposure on subsequent development of PTSD symptoms, either in the presence or absence of subsequent post-deployment stressors, was not investigated.

In the current study, we aimed to explore interaction effects of combat stress exposure, immune reactivity, and post-deployment stressful life events (SLE) on changes in PTSD symptoms after return from deployment. We investigated the production of a broad range of innate and T-cell cytokines upon stimulation, including pro- and anti-inflammatory cytokines, as well as chemokines one month following military deployment. We hypothesized that immune activation would influence change in PTSD symptoms following high combat stress exposure in response to new (i.e. post-deployment) stressful life events, given the evidence of stress sensitization in PTSD. Specifically, we predicted that high cytokine production 1 month after deployment would interact with the number of reported stressors 1 year following deployment as well as combat stress exposure, resulting in increases in PTSD levels.

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traumatic event (Elhai et al., 2009). Participants were assigned a probable PTSD diagnosis when their score on the SRIP was ≥38 (Van Zuiden et al., 2011b). This cutoff score corresponds to the mean plus two standard deviations, which coincides with the 95th percentile of scores before deployment within a population of 704 soldiers from the Dutch Armed Forces (mean: 26.91, SD = 5.34).

2.2.5. CD2/CD28-induced T-cell cytokine/chemokine production

Whole blood, diluted 1:10 with RPMI-1640 (Gibco, Grand Island, NY), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 μM L-glutamine was incubated with anti-CD2/CD28 monoclonal antibodies (CLB, Amsterdam, Netherlands, final concentration anti-CD2.1/anti-CD2.2 0.33 μg/ml and anti-CD28 1.33 μg/ml) for 72 h at 37 °C/5% CO2 in 96-well round-bottomed plates. Production of interleukin (IL)-2, IL-4, IL-5, IL-6, IL-10, TNF-α, monocyte chemoattractant protein (MCP)-1 (CCL2), interferon-gamma induced protein (IP)-10 and RANTES (CCL5) by stimulated T-cells was measured in supernatant using multiplex cytokine assay (Korenromp et al., 2011). In addition, interferon (IFN)-γ was analyzed by enzyme-linked immunosorbent assay (ELISA; CLB, Amsterdam, the Netherlands).

2.2.6. Lipopolysaccharide-induced monocyte cytokine production

Whole blood, diluted 1:10 with RPMI-1640 (Gibco, Grand Island, NY), was incubated with lipopolysaccharide (LPS; *Escherichia coli* 0127:B8, Sigma, final concentration 1 ng/ml) for 24 h at 37 °C/5% CO2 in 96-well flat-bottomed plates. Supernatant was analyzed by multiplex assay for the presence of IL-1α, IL-1β, IL-6, IL-8, IL-10 and TNF-α produced by stimulated monocytes.

2.3. Analysis

Latent growth and path analyses were performed using SPSS/Amos software versions 20.0 and 17.0, respectively, and exploratory structural equation modeling was performed using MPlus version 7.11. Missing data were handled using the full information maximum likelihood (FIML) procedure. Data screening revealed moderate to severe non-normality in PTSD symptom scores, early trauma, number of previous deployments, as well as the interaction variables (see below).

We used Bayesian statistics to estimate regression coefficients (paths) and 95% credibility intervals as well as standardized regression coefficients from our model. The rationale for applying Bayesian estimation was twofold. First, Bayesian estimation is not based on normality assumptions (Bolstad, 2004) and therefore Bayesian estimates are unaffected by nonnormality. Second, the Bayesian credible interval is intuitively interpretable because it allows direct probability statements about the parameters, i.e. the probability of a parameter being within the stated interval, unlike the confidence statements in frequentist statistics. This is therefore a compelling reason for using Bayesian statistics (Bolstad, 2004). Estimates are statistically significant at $p \leq .05$ if the 95% credible interval does not include

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zero. Bayesian estimation of the (standardized) regression coefficients does not otherwise affect their interpretation.

We used the default settings in Amos (Arbuckle, 2009), using an uninformative flat prior. The Markov Chain Monte Carlo (MCMC) sampling for the Bayesian estimation was continued until subsequent runs were sufficiently uncorrelated, i.e. when the value of the Gelman—Carlin—Rubin convergence statistic was below the conservative default value of 1.002. The Markov Chain Monte Carlo (MCMC) sampling for the Bayesian estimation was continued until subsequent runs were sufficiently uncorrelated, i.e. when the value of the Gelman—Carlin—Rubin convergence statistic was less than the conservative default value of 1.002. Bayesian model fit indices, including the posterior predictive p-value (PPP), deviance information criterion (DIC), and effective number of parameters were calculated besides maximum likelihood fit indices, including the discrepancy (χ²), comparative fit index (CFI), non-normal fit (Tucker-Lewis index (NFI/TLI)), root-mean-square error of approximation (RMSEA), and Akaike information criterion (AIC).

### 2.3.1. Exploratory structural equation modeling

Cytokine data reduction was done using exploratory structural equation modeling (ESEM), which is a recently developed statistical method in which exploratory factor analysis is performed within a structural equation modeling setting (Asparouhov and Muthén, 2009; Van Zuiden et al., 2011b). The ESEM model in the current study was the same as the model described by Van Zuiden et al. (2011b). We therefore expected the model estimates, factor scores, and model fit to be very similar to those reported in the latter study. Differences between the current study and the Van Zuiden et al. (2011b) study are as follows: (1) the present study included only cytokine production at T1, whereas the Van Zuiden et al. study used cytokine production at T0, T1, and T2; (2) the present study is based on a subsample that included all participants (N=814 at T0) who were administered a SLE checklist one year after deployment, whereas the Van Zuiden et al. study additionally included earlier recruited participants (N=209 at T0), who were not administered the SLE checklist. Cytokine data at T1 were available for N=684 participants; blood samples of 14 participants at T2 were not available due to technical and handling problems. Cytokine data were not normally distributed and therefore transformed prior to ESEM. CD2/CD28-induced cytokines were log10-transformed except IL-10 and IP-10, which were square-root transformed. Of LPS-induced cytokines, IL-1α, IL-10 and TNF-α were log10-transformed, and IL-1β, IL-6, and IL-8 were square-root transformed. Outliers were removed if z-values were outside the range of ±3.29. The number of outliers was for CD2/CD28-induced cytokines: IL-2: 2 (0.29%); IL-4: 6 (0.88%); IL-5: 8 (1.17%); IL-6: 12 (1.76%); IL-10: 4 (0.59%); TNF-α: 12 (1.76%); IFN-γ: 1 (0.16%); MCP-1: 3 (0.44%); IP-10: 3 (0.44%); RANTES: 7 (1.05%). For LPS-induced cytokines: IL-1α: 12 (1.80%); IL-1β: 2 (0.30%); IL-6: 0 (0%); IL-8: 2 (0.30%); IL-10: 7 (1.05%); TNF-α: 10 (1.50%).

### 2.3.2. Latent growth modeling and path analysis

We applied a linear latent growth model (LGM) to analyze the baseline (post-deployment) level as well as the linear change in PTSD symptoms that were assessed at T1—T4. The path diagram is presented in the upper part of Fig. 2. LGMs can be extended to include structural parameters as predictors (Duncan et al., 2006). Thus, we examined the relationship between predictor variables (i.e., combat stress exposure, post-deployment stressful life events, cytokine production, as well as their interactions), baseline level, and linear change. Two-way and 3-way interaction effects were calculated and added to the model. Following

![Path diagram of the cytokine-SLE interaction model. Unobserved variables are shown as ovals, factor loadings as arrows with fixed regression weights, covariances as curved lines, and observed variables as squares. E1 to E6 represent residual error variances. Cyt1: T-cell cytokine production; Cyt2: T-cell-induced chemokine/IL-6 production; Cyt3: innate cytokine production; Exp: combat stress exposure; PTSS: PTSD symptoms; SLE: post-deployment stressful life events.](http://dx.doi.org/10.1016/j.psyneuen.2014.07.010)
recommendations to eliminate threats to the test of overall interaction effects due to multicollinearity (Dawson, 2014; Jaccard et al., 1990), variables (specifically, combat stress exposure and post-deployment SLE) were standardized (by subtracting the sample mean from each individual raw score and then dividing the difference by the sample standard deviation) before calculating interaction effects. In model nr. 1, we included high combat stress exposure as a predictor of both baseline level and linear change in PTSD symptoms, and post-deployment SLE as well the combat stress exposure × SLE interaction as predictors of linear change in PTSD symptoms. In model nr. 2, cytokine production variables were added as a predictor of baseline level and linear change, and interactions between cytokine production and combat stress exposure were included as predictors of baseline level and linear change. Finally, the model included 3-way interaction effects between cytokine production, combat stress exposure, and post-deployment SLE on linear change in PTSD symptoms. Fig. 2 represents the full path diagram.

2.3.3. Path analysis adjusted for covariates

Because PTSD symptoms as well as cytokine production may be related to background variables, including gender, age, education, rank, the number of previous deployments, early life trauma, smoking, BMI, and use of medications (specifically, oral anti-contraceptives, non-systemic glucocorticoids, antihistamines, cholesterol-lowering and antihypertensive drugs), we specified an extended model (model 3) including these variables as covariates predicting both baseline level and linear change. All covariates were allowed to covary with all other predictors.

2.3.4. Probing three-way interaction effects

Using slope difference tests and macros provided by Dawson (Dawson, 2014) three-way interactions were probed and visualized. Variances and covariances of estimates needed to calculate slope difference tests were estimated using FIML in Amos.

3. Results

3.1. Cytokine data reduction

Descriptive analyses of sample characteristics are reported in Table 1. Descriptive analyses of the cytokine data are reported in the Supplementary Table. Table 2 shows the results of the cytokine data reduction. The data showed an adequate fit to the previously observed (Van Zuiden et al., 2011b) 4-factor model incorporating data from 9 CD2/CD28-induced and 4 LPS-induced cytokines ($\chi^2(32) = 159.36, p < .001$, CFI = 0.97, NNFI = 0.94,

<table>
<thead>
<tr>
<th>Table 1 Descriptive analyses.</th>
<th>Mean</th>
<th>(SD)</th>
<th>[Range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) during deployment</td>
<td>27.33</td>
<td>(8.16)</td>
<td>[18–60]</td>
</tr>
<tr>
<td>Number of previous deployments</td>
<td>0.84</td>
<td>(1.15)</td>
<td>[0–6]</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.58</td>
<td>(2.70)</td>
<td>[17.84–41.03]</td>
</tr>
<tr>
<td>Combat stress exposure</td>
<td>3.99</td>
<td>(2.58)</td>
<td>[0–10]</td>
</tr>
<tr>
<td>Early Trauma Inventory score</td>
<td>3.22</td>
<td>(2.91)</td>
<td>[0–17]</td>
</tr>
<tr>
<td>Stressful life events 1 year post-deployment</td>
<td>0.76</td>
<td>(0.96)</td>
<td>[0–4]</td>
</tr>
<tr>
<td>Self-Rating Inventory for PTSD total score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month after deployment</td>
<td>28.08</td>
<td>(6.43)</td>
<td>[22–61]</td>
</tr>
<tr>
<td>6 months after deployment</td>
<td>28.09</td>
<td>(7.36)</td>
<td>[22–82]</td>
</tr>
<tr>
<td>1 year after deployment</td>
<td>27.20</td>
<td>(7.08)</td>
<td>[22–86]</td>
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<tr>
<td>2 years after deployment</td>
<td>26.75</td>
<td>(6.19)</td>
<td>[22–73]</td>
</tr>
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<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>740</td>
<td></td>
<td>(90.10)</td>
</tr>
<tr>
<td>Female</td>
<td>74</td>
<td></td>
<td>(9.90)</td>
</tr>
<tr>
<td>Education</td>
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<tr>
<td>Lower</td>
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<td>(41.37)</td>
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<tr>
<td>Middle</td>
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<td>(49.32)</td>
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<tr>
<td>Higher</td>
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<td>(9.32)</td>
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<td>Rank during deployment</td>
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<tr>
<td>Soldier</td>
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<td>(43.77)</td>
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<tr>
<td>Corporal</td>
<td>168</td>
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<td>(21.13)</td>
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<td>Noncommissioned officer</td>
<td>181</td>
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<td>(22.77)</td>
</tr>
<tr>
<td>Officer</td>
<td>98</td>
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<td>(12.33)</td>
</tr>
<tr>
<td>Smokes cigarettes</td>
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<tr>
<td>Yes</td>
<td>331</td>
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<td>(45.84)</td>
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<tr>
<td>No</td>
<td>391</td>
<td></td>
<td>(54.16)</td>
</tr>
</tbody>
</table>

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RMSEA = 0.076, AIC = 16,922.77). As expected, the factor loadings were very similar to those reported previously (Van Zuiden et al., 2011b). The first exploratory factor contained CD2/CD28-induced cytokines (IL-2, IL-5, IL-6, IL-10, TNF-α, and IFN-γ) as well as the chemokines RANTES and IP-10. Because CD2/CD28 is a strong activator of T-cells, factor 1 was named T-cell cytokines. The second exploratory factor included the CD2/CD28-induced chemokines MCP-1 and RANTES and CD2/CD28 induced IL-6 and was therefore named T-cell-induced chemokines/IL-6. The third exploratory factor included all LPS-induced cytokines (IL-1α, IL-β, IL-10, and TNF-α). Since LPS stimulates the innate immune system, this factor was referred to as innate cytokines. A fourth residual factor (see Table 2) could not be interpreted in a functional way and was therefore omitted from further analyses.

### 3.2. Modeling PTSD symptoms over time

Latent growth modeling of PTSD symptoms assessed 4 times over 2 years after deployment revealed a baseline level mean SRIP score of 28.15 (SD: 5.80, 95% CI: 27.69–28.60), and a linear change of −1.11 (SD: 5.01, 95% CI: −1.69 to −0.57) over 2 years. The model fitted the data well ($\chi^2(5) = 12.72$, $p = .03$, RMSEA = .044, CFI = .99, NFI = .98, AIC = 30.72; PPP = .51, DIC = 10263.60, effective nr. of parameters = 8.30, convergence = 1.0014, samples = 500 + 16501).

Point prevalence estimates showed a gradual decline in probable PTSD prevalence over time. Specifically, probable PTSD was endorsed by $N = 64$ (9.6%) at T1, $N = 59$ (9.5%) at T2, $N = 33$ (7.2%) at T3, and $N = 22$ (5.7%) at T4. Of participants providing relevant data across at least 3 assessments ($N = 455$), $N = 374$ (82.2%) did not endorse probable PTSD at any assessment, $N = 27$ (5.9%) endorsed persistent or fluctuating probable PTSD at T1 as well as T2, T3, and/or T4, $N = 15$ (3.3%) endorsed time-limited probable PTSD at T1 only, and $N = 39$ (8.6%) endorsed late-onset probable PTSD at T2, T3, and/or T4 but not T1.

### 3.3. Predictors of linear change in PTSD symptoms over 2 years

In Table 3, path analysis results on the effects of stressor exposure and cytokine production on PTSD symptoms are presented. The models showed good fit to the data; model fit indices are also reported in Table 3. The initial model (model nr. 1) included combat stress exposure as a predictor of both baseline level and linear change. In addition, post-deployment SLE as well as the interaction between combat stress exposure and post-deployment SLE, were predictors of linear change in PTSD symptoms. As expected, combat stress exposure had significant effects on baseline level, explaining 3% of the variance in baseline level. Post-deployment SLE and the combat stress exposure*SLE interaction both had significant positive effects on linear change, explaining 7% of the variance in linear change. The cytokine model (model nr. 2) additionally included T-cell cytokine production, T-cell-induced chemokine/IL-6 production, and innate cytokine production effects, as well as interaction effects of cytokine production and combat stress exposure on baseline level and linear change. In addition, two- and three-way interaction effects of cytokine production, combat stress exposure, and post-deployment SLE on linear change in PTSD symptoms were included. Only combat stress exposure had a significant effect on baseline level; the model explained 4% of the variance in baseline level. A linear increase in PTSD symptoms post-deployment (from 1 month to 2 years) was predicted by 3-way interactions between (1) post-deployment SLE, (2) combat stress exposure, and (3) T-cell and/or innate cytokine production. These effects explained 15% of the variance in linear change. The effects of post-deployment SLE and the 'combat stress exposure x
Table 3  Effects of stressor exposure and cytokine production on PTSD symptoms: baseline level and linear change over 2 years.

<table>
<thead>
<tr>
<th>Baseline level</th>
<th>Coeff.</th>
<th>95% CI</th>
<th>Std. coeff.</th>
<th>Coeff.</th>
<th>95% CI</th>
<th>Std. coeff.</th>
<th>Coeff.</th>
<th>95% CI</th>
<th>Std. coeff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combat stress exposure</td>
<td>1.06</td>
<td>(0.56–1.54)</td>
<td>0.18</td>
<td>1.07</td>
<td>(0.56–1.56)</td>
<td>0.19</td>
<td>0.72</td>
<td>(0.21–1.23)</td>
<td>0.13</td>
</tr>
<tr>
<td>T-cell cytokines</td>
<td>0.33</td>
<td>(−0.18–0.86)</td>
<td>0.06</td>
<td>0.21</td>
<td>(−0.31–0.76)</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-cell-induced chemokines/IL-6</td>
<td>−0.08</td>
<td>(−0.62–0.46)</td>
<td>−0.01</td>
<td>−0.13</td>
<td>(−0.68–0.42)</td>
<td>−0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innate cytokines</td>
<td>0.44</td>
<td>(−0.13–1.00)</td>
<td>0.07</td>
<td>0.53</td>
<td>(−0.05–1.09)</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure × T-cell cytokines</td>
<td>0.13</td>
<td>(−0.37–0.63)</td>
<td>0.02</td>
<td>0.11</td>
<td>(−0.39–0.61)</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure × T-cell-induced chemokines/IL-6</td>
<td>0.18</td>
<td>(−0.36–0.73)</td>
<td>0.03</td>
<td>0.17</td>
<td>(−0.38–0.68)</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure × innate cytokines</td>
<td>0.15</td>
<td>(−0.41–0.71)</td>
<td>0.03</td>
<td>0.02</td>
<td>(−0.52–0.57)</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Linear change

| Combat stress exposure              | −0.02  | (−0.69–0.64) | 0.00        | −0.31  | (−1.04–0.40) | −0.06      | −0.23  | (−1.02–0.58) | −0.04      |
| Post-deployment SLE                 | 0.89   | (0.02–1.67)  | 0.18        | 0.78   | (−0.14–1.67) | 0.15      | 0.81   | (−0.11–1.66) | 0.16      |
| Exposure × SLE                      | 1.28   | (0.39–2.12)  | 0.23        | 0.89   | (−0.13–1.90) | 0.16      | 0.77   | (−0.26–1.75) | 0.13      |
| T-cell cytokines                    | −0.25  | (−0.94–0.44) | −0.05       | −0.26  | (−0.96–0.44) | −0.05     |
| T-cell-induced chemokines/IL-6      | 0.53   | (−0.16–1.22) | 0.10        | 0.65   | (−0.11–1.39) | 0.12     |
| Innate cytokines                    | −0.41  | (−1.19–0.36) | −0.07       | −0.45  | (−1.25–0.31) | −0.08     |
| Exposure × T-cell cytokines         | 0.26   | (−0.55–1.09) | 0.05        | 0.33   | (−0.47–1.12) | 0.06     |
| Exposure × T-cell-induced chemokines/IL-6 | −0.10  | (−0.91–0.70) | −0.02       | −0.06  | (−0.88–0.77) | −0.01     |
| Exposure × innate cytokines         | −0.18  | (−1.10–0.72) | −0.03       | −0.21  | (−1.08–0.61) | −0.04     |
| T-cell cytokines × SLE              | 0.53   | (−0.49–1.49) | 0.09        | 0.64   | (−0.32–1.59) | 0.11     |
| T-cell-induced chemokines/IL-6 × SLE | 0.16   | (−1.00–1.31) | 0.03        | 0.27   | (−0.90–1.40) | 0.04     |
| Innate cytokines × SLE              | 0.42   | (−0.60–1.41) | 0.08        | 0.58   | (−0.38–1.55) | 0.11     |
| Exposure × T-cell cytokines × SLE   | 1.67   | (0.30–2.94)  | 0.25        | 1.80   | (0.50–3.03)  | 0.27     |
| Exposure × T-cell-induced chemokines/IL-6 × SLE | −1.00  | (−2.29–0.40) | −0.14       | −1.10  | (−2.37–0.19) | −0.16     |
| Exposure × innate cytokines × SLE   | 1.33   | (0.26–2.31)  | 0.23        | 1.38   | (0.40–2.32)  | 0.24     |

Note: Values are regression coefficients. Interactions between variables are denoted with a multiplication sign ("×"). CI: credible interval; SLE: stressful life events; Std.: standardized. Values in bold are statistically significant (p < .05).

a For the model: $\chi^2(13) = 35.13$, p = .00, RMSEA = .05, NNFI = .94, AIC = 79.13; PPP = 0.50, DIC = 11,452.21, effective number of parameters = 21.65, convergence = 1.0016, samples = 500 × 39,501.

b For the model: $\chi^2(43) = 69.44$, p = .01, RMSEA = .03, CFII = .98, NNFI = .92, AIC = 401.44; PPP = 0.50, DIC = 15,815.45, effective number of parameters = 153.89, convergence = 1.0019, samples = (500 + 66,501) × 8.

c For the model: $\chi^2(59) = 91.97$, p = .004, RMSEA = .03, CFII = .99, NNFI = .92, AIC = 841.97, Pp = .50, DIC = 20,899.78, effective number of parameters = 312.68, convergence = 1.0018, samples = (500 + 67,501) × 16.
SLE’ interaction on linear change were no longer significant in model 2.

In order to control for background variables, including gender, age, education, rank, the number of previous deployments, early life trauma, smoking, influencing medications, and BMI, we replicated the path analysis using an extended model (model nr. 3) including these variables as covariates. The results of this analysis were very similar to the unadjusted results (see Table 3). Early life trauma had a significant effect on baseline level (unstandardized coefficient = 0.48 (95% CI, 0.31–0.65), standardized coefficient = 0.24), but not on linear change. None of the other covariate effects was significant (data not shown). The adjusted results are shown in Table 3. The adjusted model explained 11% of the variance in baseline level, and 18% of the variance in linear change. To ascertain that none of the reported results were dependent on inclusion of the small number of women, we repeated the analysis of the LGM as well as the initial and cytokine models with gender included as a covariate. All these three models showed a good fit to the data and yielded virtually identical results to those already reported, including no significant effects of the gender covariate in any of the models (data not shown).

3.4. Probing interaction effects

Figs. 3 and 4 show interaction plots of the significant 3-way interaction effects of post-deployment SLE, combat stress exposure, and cytokine production (T-cell and innate, respectively) on linear change in PTSD symptoms. In the presence of high combat exposure and high T-cell or innate cytokine production, post-deployment stressors exerted large effects on linear change in PTSD symptoms. There was a decrease in symptoms with low post-deployment stressors, and an increase in symptoms with high post-deployment stressors. These effects ranged from a decrease of 6 points to a 2-point increase, a range of 8 points on the self-report inventory of PTSD. This equals 1.6 standard deviations of the linear change in PTSD symptoms from 1 month to 2 years.

Post hoc slope difference tests revealed significant differences in effects of post-deployment SLE on change in PTSD symptoms between the groups with both high cytokine production and high combat stress exposure and the other groups, i.e., those with either low cytokine production or low exposure. Significant differences were observed between the group with high T-cell cytokines, high exposure and the group with (1) high T-cell cytokines, low exposure: $t = 4.20, p < .001$; (2) low T-cell cytokines, high exposure: $t = 2.91, p = .004$; and (3) low T-cell cytokines, low exposure: $t = 2.37, p = .018$. In addition, significant differences were found between the group with high innate cytokines, high exposure and the group with (1) high innate cytokines, low exposure: $t = 3.57, p < .001$; (2) low innate cytokines, high exposure: $t = 2.69, p = .007$; and (3) low innate cytokines, low exposure: $t = 2.41, p = .016$.

4. Discussion

This study showed that only in soldiers reporting high levels of exposure to combat, the effects of stressful life events during the first year after return from military deployment on the course of symptoms of PTSD are moderated by immune reactivity, as measured by the capacity of monocytes and T-cell lymphocytes to produce cytokines upon stimulation one month after deployment. Specifically, after high combat stress exposure, high mitogen-stimulated T-cell cytokine production and high innate cytokine production predict a steeper increase in PTSD symptoms over 2 years following return from deployment in response to post-deployment SLE. These results imply that effects of post-deployment SLE on the course of PTSD symptoms after return from deployment largely depend on combat stress exposure as well as immune reactivity following return from deployment. This is consistent with a concept of symptoms...
of PTSD as indicative of increased sensitivity to stressful stimuli after exposure to a traumatic event, i.e. stress sensitization (Fries et al., 2005; Heim et al., 2000; Smid et al., 2012, 2013). However, our results suggest that high combat exposure does not by itself lead to increased sensitivity to post-deployment SLE, but only in the presence of immune activation as evidenced by high T-cell and innate cytokine production. Also, immune activation by itself is not sufficient to lead to increased reactivity to SLE, but only following high combat stress exposure.

Causes of high T-cell and innate cytokine production may be related to the post-deployment state as well as to pre-existent traits. Soldiers who returned from deployment were exposed to a variety of factors that are likely to have strong immunological effects beyond possible antigenic stimulation, including combat stressors, chronic stress, exertion, weather extremes, and sometimes injury. We (Van Zuiden et al., 2011b) reported an increase in stimulated T-cell cytokine production in soldiers from pre-deployment to 6 months post-deployment, indicating that deployment to a combat-zone increases the capacity of T-cells to produce cytokines until at least 6 months after return; conversely, innate cytokine production after stimulation with a mitogen decreased during this interval. These findings could imply a direct effect of military deployment on cytokine production.

However, high combat stress exposure does not always coincide with high cytokine production, and thus, pre-existent traits may also be involved. Immunological trait aspects may include genetic make-up and epigenetic programming after earlier stress, trauma, and other immunological and/or neuroendocrinological challenge. Although immunologic responses are obviously variable over time, a study (ter Wolbeek et al., 2007) employing repeated assessments of stimulated cytokine production in fatigued adolescents (not exposed to chronic or severe stress) showed that the within-subject ranks relatively to the rest of the participants were fairly stable over time. Hypocortisolism associated with PTSD may lead to increased cytokine production (Raison and Miller, 2003; Rohleder et al., 2004) and has been suggested to comprise a pre-existent vulnerability trait. Specifically, children of parents with PTSD exhibited hypocortisolism and were at increased risk of developing PTSD themselves following trauma-exposure (Yehuda et al., 2007). However, several studies did not find a predictive effect of low circulating cortisol prior to exposure to traumatic events on the eventual development of PTSD symptoms following exposure (for a review, see Van Zuiden et al., 2013). Alternatively, interaction effects of pre-existent trait vulnerabilities and aspects of the post-deployment state may be implicated in the development of high innate cytokine production after deployment.

Our finding that both increased cytokine production by innate and adaptive cells may be associated with PTSD symptoms is interesting in the light of earlier literature suggesting that an overactive innate immune system may lead to a suppressed adaptive immune system (Raison and Miller, 2003). In the current study, increased innate and T-cell cytokine production appear to operate in tandem, suggesting interactions between the adaptive and innate immune systems. It may be hypothesized that our earlier observation that high T-cell cytokine production before deployment predicts high levels of depression 6 months after deployment (Van Zuiden et al., 2011b) may result from a low capacity of glucocorticoids to regulate T-cell activation in these individuals. Indeed, the sensitivity of T-cells but not of monocytes for glucocorticoids prior to deployment has been found to be a predictive factor for the development of high levels depression symptoms with or without comorbid PTSD symptoms in response to deployment (Van Zuiden et al., 2012).

In a recent systematic review of chemokines in psychiatric disorders, no associations between chronic stress and chemokines were reported, but several chemokines were associated with psychopathology across different psychiatric disorders (Stuart and Baune, 2014). Our results do not suggest a role of chemokines in the development of stress related symptoms early after traumatic stress events.
exposure. However, these findings do not preclude associations between chemokine production and psychopathology later on.

Besides pro-inflammatory activity, some of the cytokines included in both T-cell and innate cytokine factors of our ESEM model (Van Zuiden et al., 2011b) are characterized by anti-inflammatory activity. Specifically, IL-2, TNF-α, and IFN-γ are pro-inflammatory T-cell cytokines, and IL-1α, IL-1β, IL-6, IL-8, and (again) TNF-α are innate (monocyte-produced) pro-inflammatory cytokines. In contrast, IL-4, IL-5, and IL-10 are anti-inflammatory T-cell cytokines, and IL-10 is also an innate anti-inflammatory cytokine. Both pro- and anti-inflammatory cytokines are produced in response to and in vitro stimulation. In vivo, the balance between pro- and anti-inflammatory cytokine production determines whether a pro-or anti-inflammatory milieu ensues. Thus, both pro- and anti-inflammatory cytokines may be involved in the development of PTSD symptoms in stress sensitized soldiers, similar to previous findings with regard to depression symptoms (Van Zuiden et al., 2011b). Alternatively, increases in anti-inflammatory cytokines may compensate increases in pro-inflammatory cytokines or vice versa without contributing to the development of PTSD symptoms.

One way in which enhanced immunity could contribute to the development of PTSD is through cytokine signaling in conjunction with production of reactive oxygen species (ROS) in regions of the brain commonly associated with PTSD, including the hippocampus, amygdala, and pre-frontal cortex. A recent study using a predator exposure/psychosocial stress animal model of PTSD (Wilson et al., 2013) found pro-inflammatory cytokines and ROS were elevated in all these three regions of the rat brain, indicating increased oxidative stress and inflammation. In addition, oxidative stress and inflammation were elevated systemically, as evidenced by increased ROS and pro-inflammatory cytokines in the adrenal glands and circulating blood (Wilson et al., 2013).

Our findings of a strong linear decrease in PTSD symptoms in highly exposed, high T-cell/innate cytokine producing soldiers in the presence of low post-deployment SLE (see Figs. 3 and 4) suggest both beneficial and detrimental effects of high cytokine production, depending on the subsequent occurrence of SLE. A systemic model of the moderating role of immune processes on learning and memory has recently been proposed, which specifies both beneficial and detrimental effects of immune system activation on behavioral and neural plasticity (Yirmiya and Goshen, 2011). In circumstances of low stress, memory consolidation is dependent on the production of specific cytokines, such as IL-1, IL-6, and TNF-α, chemokines and immune mediators in the hippocampus, hypothalamus, and brain stem (Vitkovic et al., 2000; Yirmiya and Goshen, 2011).

Following exposure to severe psychological stress, several components of the immune system are stimulated, in which IL-1 plays an important role (Goshen and Yirmiya, 2009), resulting in increased expression of pro-inflammatory cytokines in various brain areas, including the hippocampus, hypothalamus and brain stem (Maier, 2003; Yirmiya and Goshen, 2011). In addition, peripheral immune cells produce pro-inflammatory cytokines, which influence various brain regions through humoral and neural pathways. High levels of pro-inflammatory cytokines associated with injury, inflammation, and severe psychological stress have direct detrimental effects on memory functioning and neural plasticity (Yirmiya and Goshen, 2011).

Strengths of the current study include the prospective design, the comprehensive evaluation of stimulated cytokine and chemokine production, as well as the duration of follow-up covering two years following return from deployment. Some methodological limitations to this study must be acknowledged. First, the PTSD symptoms as well as stressor exposure were assessed by self-report questionnaire. The stressor exposure questionnaires were specifically designed for this study, and thus not validated in independent samples. Use of interview assessment of PTSD symptoms and exposure to stressful life events could be considered more valid. However, given the large size of our sample, paper and pencil assessments were more feasible than interviews, and the SRIP has demonstrated concurrent validity with diagnostic interviews (Hovens et al., 2002). Second, differential attrition in our study represents another potential limitation. Importantly, symptoms of posttraumatic distress at the initial assessment did not predict attrition, and our adjusted analyses suggested that there were no meaningful effects of differential attrition on our results. Third, cytokine dysregulation may affect the development of depressive symptoms that were not accounted for in this study, and depression symptoms and PTSD symptoms are often comorbid. Future research may extend previous findings (Van Zuiden et al., 2011b) on the effects of dysregulated cytokine production after combat stress exposure on development and course of depression symptoms. Finally, although the effects of high combat exposure, high cytokine production, and post-deployment SLE on PTSD symptom change were considerable, their clinical relevance has not yet been established and therefore requires further investigation.

Our findings have several implications for research and practice. High combat exposed soldiers, their families and those professionally involved in their well-being may usefully learn to recognize increased responsiveness to post-deployment stressful life events. Early interventions may be targeted at high risk groups based on both combat exposure and neurobiological risk profiles that may include measures of immune activation. Foreseeable stressors and resource losses, including unemployment and physical impairments, may be an effective target for secondary prevention of psychological distress. Our data provide support for efforts to normalize immune reactivity by pharmacological means (Jones and Thomsen, 2013). Normalization of cytokine production may be achieved using a panoply of agents, including biological response modifiers (e.g. cytokine antibodies), cyclooxygenase inhibitors (e.g. celecoxib), and antioxidants (e.g. N-acetyl)cysteine) (Haroon et al., 2012; Li et al., 2011). Clearly, more research is needed before the clinical merits of these agents in soldiers returned from deployment can be evaluated.

Behavioral factors that may be amenable to intervention and that can contribute to normalization of immune functioning include diet, smoking, coffee, alcohol, exercise, and sleep (O’Connor and Irwin, 2010). State versus trait aspects of high cytokine production may comprise an important avenue for further scientific exploration. This may include analyses on the role of early life trauma as a moderating...
trait variable in stress sensitization following adult exposure to trauma. Finally, it is warranted that future studies of PTSD include assessments of stressful life events following the traumatic event.

Conflicts of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.07.010.

References


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Cytokine production underlying stress sensitization


