Hypocortisolism and Increased Glucocorticoid Sensitivity of Pro-Inflammatory Cytokine Production in Bosnian War Refugees with Posttraumatic Stress Disorder

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Background: *Posttraumatic stress disorder (PTSD) is associated with dysregulation of the hypothalamus pituitary adrenal (HPA) axis. Alterations include various responses to HPA axis stimulation, different basal hormone levels, and changes in glucocorticoid receptor (GR) numbers on lymphocytes. The functional significance of these latter changes remains elusive.*

Methods: *Twelve Bosnian war refugees with PTSD and 13 control subjects were studied. On 2 consecutive days, they collected saliva samples after awakening and at 11, 15, and 20 hours. Glucocorticoid (GC) sensitivity was measured by dexamethasone (DEX) inhibition of lipopolysaccharide (LPS)-induced interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) production in whole blood.*

Results: *The PTSD patients showed no cortisol response after awakening and had lower daytime cortisol levels ($F = 14.57$, $p < .001$). Less DEX was required for cytokine suppression in PTSD patients (IL-6: $t = -2.82$, $p = .01$; TNF-α: $t = 5.03$, $p < .001$), reflecting higher GC sensitivity of pro-inflammatory cytokine production. The LPS-stimulated production of IL-6, but not TNF-alpha, was markedly increased in patients (IL-6: $F = 10.01$, $p < .004$; TNF-α: $F = .89$, $p = .34$).*

Conclusions: *In refugees with PTSD, hypocortisolism is associated with increased GC sensitivity of immunologic tissues. Whether this pattern reflects an adaptive mechanism and whether this is sufficient to protect from detrimental effects of low cortisol remains to be investigated.*

Key Words: Cytokines, glucocorticoid sensitivity, HPA axis, hypocortisolism, inflammation, posttraumatic stress disorder

Psychosocial stress is a strong activator of the hypothalamus pituitary adrenal (HPA) axis, leading to increased levels of cortisol, which exerts a large array of effects on most bodily systems (Chrousos and Gold 1992). These short-term increases of peripheral glucocorticoid (GC) levels are believed to be protective against the effects of other stress-activated bodily systems (Munck et al 1984).

Persistence of stress over longer periods of time, including chronic stress such as unemployment (Ockenfels et al 1995) or burnout (Pruessner et al 1999) or that following traumatic events such as war, sexual abuse, or motor vehicle accidents, is believed to induce long-term alterations of the HPA axis (Heim et al 2000; Yehuda et al 1990). Similar to patients with major depressive disorder (MD), patients suffering from posttraumatic stress disorder (PTSD) show HPA axis alterations that indicate a chronically activated stress response, with increased corticotropin-releasing hormone (CRH) measured in cerebrospinal fluid (CSF; Baker et al 1999; Bremner et al 1997; Plotsky et al 1998), and sometimes increased norepinephrine levels in the CSF (Geracioti et al 2001).

Abnormalities of the HPA axis are less consistent but seem to differ from those observed in major depression: corticotropin (ACTH) responses to CRH stimulation are reported to be unchanged, higher, or lower compared with those of control subjects (Kellner et al 2003; Rasmusson et al 2001; Smith et al 1989). Some studies found decreased levels of cortisol in urine collected over 24 hours (e.g., Mason et al 1986; Yehuda et al 1991) and in blood plasma taken repeatedly over 24 hours (e.g., Yehuda et al 1994, 1996). Yehuda et al (1996) also pointed to a disturbed circadian rhythm of cortisol secretion, with normal levels in the morning and lower than normal levels in the evening. Others have not found differences in 24-hour urinary cortisol between patients and control subjects (Mason et al 2002), and some have even reported higher levels in PTSD (Lemieux and Coe 1995; Pitman and Orr 1990).

Furthermore, the feedback regulation of the HPA axis appears to be altered in patients suffering from PTSD. Studies employing low-dose dexamethasone (DEX) showed a stronger suppression compared with healthy control subjects (Stein et al 1997; Yehuda et al 1993b, 1995), but no differences were found in an earlier study using a higher dose of DEX (Kudler et al 1987). These findings of enhanced feedback sensitivity in PTSD seem to be in line with reports of higher numbers of glucocorticoid receptors (GR) in peripheral blood lymphocytes (Yehuda et al 1993a, 1997).

Although both stress systems (i.e., the HPA axis and the noradrenergic system) seem to be hyperactive at the central level, the picture in the periphery is less clear. Some, but not all, studies have reported a peripheral hypocortisolism, and data on catecholamines, although inconsistent as well, point to a higher activity, reactivity, or both (Bremner et al 1996; Southwick et al 1997).

Hypocortisolism may be a relevant factor in the pathogenesis of stress-related bodily disorders (Heim et al 2000), especially considering a possibly overactive noradrenergic system, which, in combination could be classified as a condition of allostatic load (McEwen and Stellar 1993). The mechanism by which hypocortisolism contributes to bodily disorders has not been completely resolved thus far. Glucocorticoids exert their major

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effects by binding to cytosolic receptors in their respective target cells. The numbers and affinities of these receptors vary greatly among tissues and organs throughout the body (Bamberger et al 1996; Schmidt and Meyer 1994). One possible mechanism could be overactivity of the immune system or, more precisely, the inflammatory response related to decreased levels of suppressive GCs.

Immune-system functioning in PTSD patients has been investigated in a number of studies. Most of these have reported increased levels of circulating pro-inflammatory cytokines (IL-6, IL-1, and TNF-α; Dekaris et al 1993; Maes et al 1999; Spivak et al 1997) and decreased levels of IFN-γ (Dekaris et al 1993), and one study reported increased IL-6 levels in CSF, but not plasma, of patients with PTSD (Baker et al 2001). Studies on immune cell phenotypes also point to increased activity in PTSD patients (Dekaris et al 1993; Sabioncello et al 2000; Wilson et al 1999), which is further underlined by findings of higher activity of cell-mediated immunity measured by standard antigen skin tests (Boscarino and Chang 1999b; Watson et al 1993) and higher natural killer (NK) cell activity (Laudenslager et al 1998). Kawamura et al (2001) report lower NK cell activity, lower T lymphocyte numbers, and lower IFN-γ production. Taken together, data on immune-system functioning in PTSD is ambiguous at present; however, a cautious conclusion could be drawn that unspecific immunity and inflammation may be overactive, whereas adaptive immunity seems to be unchanged or even depressed.

Increased activity of the inflammatory system in PTSD patients could have negative implications for somatic health and for well-being, mediated by increased activity of pro-inflammatory cytokines (Watkins and Maier 1999). In this study, we set out to investigate whether Bosnian war refugees with a PTSD diagnosis, compared with healthy control subjects, show hypocortisolism, whether these patients show an overactive inflammatory response, and whether GCs are effective in suppressing this inflammatory response. We investigated these issues by measuring the cortisol response to awakening, circadian cortisol profiles, the stimulated ex vivo production of pro-inflammatory cytokines, as well as the GC sensitivity of stimulated cytokine production.

Methods and Materials

Subjects

We investigated 12 Bosnian patients with PTSD (5 women, 7 men; mean age: 42.7, SD = 9.98; range = 30–64) and 13 age- and gender-matched healthy control subjects (8 women, 5 men; mean age: 45.7, SD = 12.79; range = 29–68). Five of the control subjects were Bosnian refugees without PTSD; eight subjects were recruited from the laboratory staff. The purpose and methods of the study were fully explained to study participants. Bosnian participants were informed by their psychotherapist (JA, see acknowledgment) and a co-therapist and translator (LJ) and received written information in Bosnian. The study protocol was approved by the ethics committee of the University of Düsseldorf. After complete description of the study, written informed consent was obtained from participants.

Twenty Bosnian refugees were asked to participate in the study. Eighteen were in psychotherapy treatment in the Psychosocial Center of Refugees in Düsseldorf (PSC) at the time of the investigation and two reported to the PSC for other reasons. Three patients from the psychotherapy groups refused participation in the study. The remaining refugees were interviewed at the PSC by the psychotherapist (psychologist) and co-therapist (physician and interpreter). In the initial semistructured interview, information on demographic data and war-related stressful life events were obtained according to the traumatic experience section of the Harvard Trauma Questionnaire (HTQ; Mollica et al 1992).

If refugees were exposed to a traumatic event, PTSD symptoms according to DSM-IV criteria were assessed using the International Diagnostic Checklist based on DSM-IV criteria for PTSD (American Psychiatric Association 1994). First, the subjects were interviewed for the presence of recurrent and intrusive recollections or dreams of any traumatic event according to DSM-IV criteria. Patients were then interviewed concerning other symptoms related to PTSD, depression, substance abuse, smoking, somatic illness, and prescribed medication. Patients who reported substance abuse were excluded from the study; all patients were free of medication and medically healthy. Two of the patients showed symptoms of comorbid depression. Three refugees did not fulfill all PTSD criteria, that is, they displayed no symptoms of criterion D (increased arousal) and less than three symptoms of criterion C (avoidance and numbing). They were therefore included in the control group. Finally, each subject completed the Symptom Checklist 90-R (SCL-90R; Derogatis and Cleary 1977) and a 30-item section of the HTQ, consisting of 16 items on DSM criteria for PTSD (reexperiencing, avoidance, and arousal symptoms) and 14 additional "refugee items." The participants were asked whether they had suffered in the past week from one or more of the 30 symptoms listed. The HTQ is a psychiatric screening instrument developed to assess posttrauma symptoms in diverse cultural contexts (Fawzi et al 1997; Mollica et al 1999). The instrument has been widely translated and used in a number of studies among various cultural groups and validated against clinical diagnosis (Mollica et al 2001). Translation into Bosnian was provided by one of the investigators (LJ) and discussed with a team of Bosnian clinicians following the standard criteria of cross-cultural research (Flaherty et al 1988; Westermeyer 1985). Back translation by another qualified Bosnian–Croatian interpreter from PSC was used to check accuracy.

German control subjects were screened for traumatic events and any past or current psychiatric or medical disorders using the German Version of Harvard Trauma Questionnaire (HTQ), the German version of Symptom Checklist—90 R (SCL-90R), and an unstructured exploratory interview. All German control subjects were medication free, and without health problems. None of the German control subjects had been previously exposed to a traumatic event according to DSM-IV criteria, and no symptoms of major depression were reported.

HPA Axis Activity

To assess HPA axis activity, PTSD patients and control subjects obtained saliva samples using the Salivette device (Sarstedt, Rommelsdorf, Germany) on 2 consecutive days to assess the cortisol response to awakening and obtain a daytime cortisol profile. Saliva sampling was done at the following time points: for the cortisol response to awakening, all participants obtained the first saliva sample immediately after awakening and then additional samples at 30, 45, and 60 min. This has been reported to be a reliable diagnostic marker for adrenocortical activity and to be altered by chronic stress and burnout (Pruessner et al 1997, 1999). A short daytime profile of cortisol secretion was obtained from additional saliva samples at 11 AM, 3 PM, and 8 PM. Free cortisol levels in saliva were measured using a

time-resolved immunoassay with fluorometric detection as described elsewhere (Dressendörfer et al 1992). For each sampling point, cortisol levels were averaged for the 2 consecutive sampling days. Self-reports of wake-up times were recorded to control for any influence of this factor.

Additionally, all subjects and patients were asked to collect 12-hour urine samples for assessment of urinary cortisol, epinephrine, and norepinephrine. Urine was collected from 7 PM to 7 AM, preserved with ethylenediamine tetraacetate (EDTA) and sodiummetabisulfate, and stored at –20°C. Analysis of cortisol, epinephrine, and norepinephrine were performed by high-pressure liquid chromatography (HPLC) as described elsewhere (Kudielka et al 1998).

GC Sensitivity Assay

Blood collection for the ex vivo GC sensitivity assay was done at one of the regular support group meetings for PTSD patients and Bosnian control subjects and in the laboratory for the control subjects (samples were obtained between 9 AM and 1 PM). Using heparinized syringes (Braun, Melsungen, Germany), 5 mL of venous blood were drawn for the whole blood assay, and 2.7 mL of blood were drawn into EDTA monovettes (Sarstedt, Rommelsdorf, Germany) for a differential blood count. All blood samples were stored at room temperature during transportation to the lab. To avoid variation due to differences in duration of transport, all blood samples were stored for 90 min before further processing.

Upon arrival at the laboratory, a differential blood count was done using an AcT Diff Cell counter (Beckman-Coulter, Krefeld, Germany) to obtain absolute numbers of monocytes, lymphocytes, and granulocytes. Heparinized whole blood of each subject or patient was diluted 10:1 with saline and divided into six 400-μL aliquots. All six aliquots were incubated with equal amounts of the bacterial endotoxin lipopolysaccharide (LPS, *Escherichia coli,* Sigma, Deisenhofen, Germany) to stimulate pro-inflammatory cytokine production by monocytes. Additionally, each aliquot was co-incubated with various concentrations of dexamethasone (DEX) on 24-well cell culture plates (Greiner, Nuertingen, Germany). The final concentrations in the respective well were 30 ng/mL LPS, and 0, 10^{-9}, 10^{-8}, $5*10^{-8}$, 10^{-7}, 10^{-6} M DEX, respectively. After 6 hours of incubation at 37°C and 5% CO_2, the plates were centrifuged for 10 min at 2000g and 4°C and the plasma supernatant was collected and stored at −80°C until analysis at completion of the study.

Concentrations of IL-6 and TNF-α were measured by commercial enzyme linked immunosorbent assay (ELISA; BD Pharmingen, San Diego, California). The detection limit of the ELISAs were 4.7 pg/mL for IL-6 and 7.8 pg/mL for TNF-α. The quality control parameters of the ELISAs were as follows: intraassay coefficient of variation (CV), 2.0–3.9%; interassay CV, 4.1–9.3%. Plates were read by a microplate reader (Anthos HTII, Anthos Labtec, Salzburg, Austria), and absorbance was transformed to cytokine concentration (ng/mL) using a standard curve computed by Anthos Winread 2.3 software (Anthos Labtec). Cytokine levels were corrected for number of monocytes in the respective blood sample because monocytes are the main source of IL-6 and TNF-α in LPS-stimulated whole blood (Berczi 1998; Elenkov et al 2001). Cytokine levels are expressed as ng/10^6 monocytes.

As an index for GC sensitivity we calculated the inhibitory concentration 50 (IC_{50}) of each individual dose-response curve for DEX inhibition of LPS-induced cytokine production. The IC_{50} reflects the specific DEX concentration required for 50% inhibition of the maximum cytokine production observed after LPS-stimulation without DEX. The IC_{50} is inversely related to GC sensitivity of the respective cytokine production.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Science Version 10.1 (SPSS Institute, Chicago, Illinois). Differences in free cortisol levels between patients and control subjects after awakening were calculated by a one-way repeated-measures analysis of variance (ANOVA) with four within-group levels. Repeated-measures ANOVA with three within-group levels was used to compute differences in the subsequent circadian profile of cortisol secretion. As additional indices for HPA axis activity, we calculated the area under the curve (AUC) of the four postawakening cortisol levels using the trapezoid formula (Pruessner et al 2003) and the mean increase of cortisol. Group differences in dose-response curves of cytokine production were calculated by repeated-measures ANOVA with six within-group levels representing the six DEX concentrations. Greenhouse-Geisser corrections for repeated measures were calculated where appropriate (indicated by decimal degree of freedom values). Student's t tests were used to assess group differences in IC_{50} levels, numbers of peripheral blood cells, AUC and mean increase of cortisol response to awakening, and urinary hormone levels. All data were tested for normality before statistical analysis using the Kolmogorov–Smirnov test. Equality of variances was tested using Levene's test. Values of $p < .05$ were considered significant.

Results

Psychological and Demographic Parameters

There were no significant differences between the PTSD and control groups in age [PTSD: 42.73 years ± 9.98 SD; control subjects: 45 years ± 12.33 SD; $t(23) = -4.80$; $p = .64$], and weight [PTSD: 76.25 kg ± 8.07 SD; control subjects: 72.31 kg ± 9.17 SD; $t(23) = 1.14$; $p = .27$]. Six of the patients (50%) and five of the control subjects (38.46%) were regular smokers (i.e., they indicated smoking more than 10 regular cigarettes or more than 20 "light" cigarettes per day).

In the SCL-90R, the PTSD group had a mean GSI score of 1.94 ± .50 SD compared with .58 ± .76 SD in the control group [$t(23) = 4,93$; $p < .001$]. In the PTSD group, the highest scores were found on the following subscales: anxiety (2.55 ± .93 SD), paranoid ideation (2.33 ± .71 SD), interpersonal sensitivity (2.11 ± .9 SD), somatization (2.08 ± .72 SD), and depressivity (1.99 ± .82 SD). In the HTQ, refugees with PTSD reported a mean number of seven experienced traumatic events. The mean number of events in the control group was .9. Among the most frequently reported events in the PTSD group were separation from family members (12), coming close to death (11), lack of food or water (10), and witnessing the murder of a stranger or strangers (10). Seven of the PTSD subjects reported lack of shelter, five were imprisoned, five had experienced the killing of a family member or friend, four had experienced torture, and four had combat experience. All refugees were subjected to a similar period of war in their home country.

Endocrine Parameters

Mean salivary cortisol levels after awakening were significantly lower in PTSD patients compared with control subjects [group effect: $F(1,23) = 14.57$, $p < .001$]. As shown in Figure 1, the baseline cortisol levels immediately after awakening did not differ between patients and control subjects, whereas the typical increase 30 min after awakening was not present in PTSD

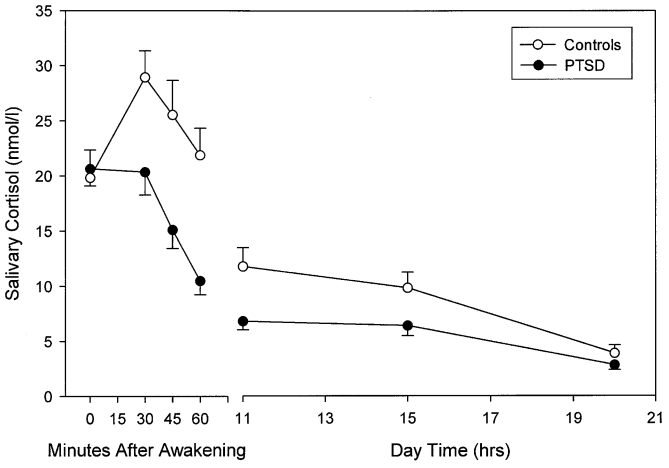

Figure 1. Cortisol response to awakening and short daytime cortisol levels in patients with posttraumatic stress disorder (PTSD) and in control subjects.

patients [group-by-time interaction: $F(1.93,42.35) = 2.776$, $p < .048$]. To exclude the possible impact of differing wakeup times, mean wakeup time were included as a covariate. Wakeup time had no significant impact [$F(1,23) = .98$; $p = .33$]. As shown in the circadian profile, the large differences between patients and control subjects sustained throughout the day [group effect: $F(1,23) = 5.33$, $p < .031$]. The AUC and mean increase of the cortisol response to awakening were also significantly lower in PTSD patients [$t(23) = -3.52$, $p < .002$; $t(23) = -2.14$, $p < .044$, respectively, data not shown].

There was no difference in 12-hour night-time urinary cortisol excretion between patients and control subjects [$t(23) = .08$, $p = .93$]. As shown in Figure 2, urinary catecholamine excretion did not differ significantly between PTSD patients and control subjects [epinephrine: $t(23) = 1.26$, $p = .22$; norepinephrine: $t(23) = 1.59$, $p = .13$].

Glucocorticoid Sensitivity

The differential blood count revealed higher levels of granulocytes in PTSD patients compared with control subjects [$t(23) = 2.81$, $p = .01$], whereas no significant differences were found in monocyte and lymphocyte numbers [$t(23) = -.57$, $p = .571$; $t(23) = 1.61$, $p = .121$, respectively].

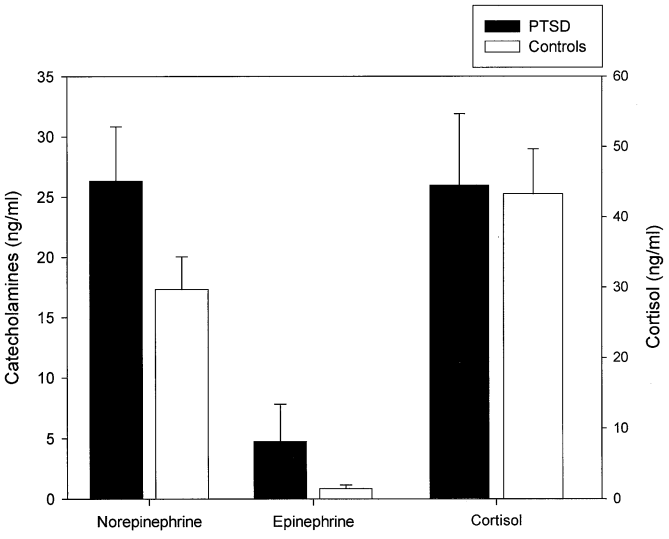

Figure 2. Catecholamines and cortisol measured in 12-hour nighttime urine in patients with posttraumatic stress disorder (PTSD) and in control subjects.

The PTSD patients showed significantly higher LPS-stimulated production of the pro-inflammatory cytokine IL-6, expressed as cytokine concentration relative to 1 million monocytes [group effect: $F(1,23) = 10.01$, $p < .004$], whereas no differences were observed for TNF-α [group effect: $F(1,23) = .89$, $p = .34$]. Coincubation of LPS-stimulated whole blood with dexamethasone-induced dose-dependant suppression of pro-inflammatory cytokine production [DEX effect: IL-6: $F(1.47,33.81) = 247.78$, $p < .0001$; TNF-α: $F(1.31,30.03) = 190.32$, $p < .0001$]. The slope of the dose-response curve indicates different sensitivities of PTSD patients compared with control subjects to the suppressive effects of DEX on LPS-induced cytokine production [group-by-DEX interaction: IL-6: $F(1.47,33.81) = 13.52$, $p < .001$; TNF-α: $F(1.31,30.03) = 3.823$, $p < .048$; see Figures 3A and 4A].

Differences in GC sensitivity were further confirmed by analysis of the IC_{50} values. The IC_{50} of the dose-response curves serves as an index for and is inversely related to GC sensitivity. As shown in Figures 3B and 4B, respectively, PTSD patients had significantly lower IC_{50} levels for both IL-6 and TNF-α suppression by DEX [IL-6: $t(23) = -2.82$, $p < .01$; TNF-α: $t(23) = -5.03$, $p < .0001$], indicating higher sensitivities of IL-6 as well as of TNF-α production toward the suppression by GCs in LPS-stimulated whole blood.

Correlational analyses showed that GC sensitivity of cytokine production is inversely related to the cortisol response to awakening (indicated by positive correlation of the IC_{50} of TNF-α suppression with the AUC of the cortisol awakening response: $r = .51$; $p = .011$; IC_{50} of IL-6 suppression: $r = .38$; $p = .07$; data not shown).

Discussion

This study clearly shows a blunted free cortisol response to awakening in Bosnian war refugees with PTSD compared with control subjects without PTSD, as well as lower cortisol levels throughout the day. Furthermore, we have shown that PTSD patients display an increased sensitivity of pro-inflammatory cytokine production, together with higher cytokine producing capacity in peripheral blood monocytes. A higher GC sensitivity seems to be associated with a lower cortisol response to awakening. These data are consistent with previous results by Yehuda et al (1996) of lower peripheral cortisol levels. In contrast to these findings, the greatest differences between patients and control subjects were found in the early morning and throughout the day, whereas the evening levels showed smaller differences. Hormones measured in urine collected overnight do not show any significant group differences. The obvious discrepancy between lower cortisol in saliva of patients and equal levels in urine leave open the question of whether methodologic issues (e.g., compliance of subjects) must be accounted for or whether night-time cortisol excretion simply may not reflect the changes seen here during the active period. Our finding of increased sensitivity of pro-inflammatory cytokines to the suppressive effects of GCs seems to be in line with previous studies showing higher numbers of GR on peripheral lymphocytes in PTSD patients (Yehuda et al 1991, 1993a).

Our findings of an increased cytokine production capacity in monocytes point to a chronic activation of the inflammatory response in PTSD patients. This is in line with studies showing increased systemic levels of pro-inflammatory cytokines (Dekaris

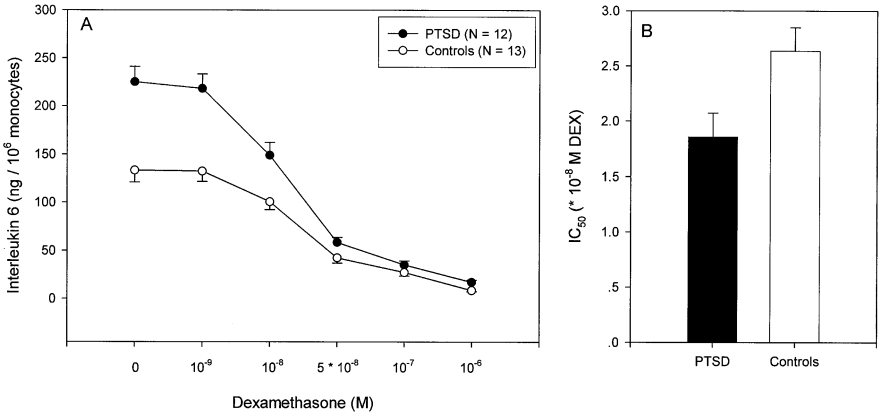

Figure 3. (A) Dexamethasone (DEX) inhibition of lipopolysaccharide-stimulated interleukin 6 (IL-6) production and **(B)** inhibitory concentration 50 (IC_{50}) as index for glucocorticoid sensitivity of stimulated IL-6 production in patients with posttraumatic stress disorder (PTSD) and in control subjects.

et al 1993; Maes et al 1999; Spivak et al 1997). Although Baker et al (2001) reported conflicting results of unchanged IL-6 levels in blood plasma, they showed in the same study that CSF levels of IL-6 were higher in PTSD patients compared with control subjects.

A chronically activated inflammatory response has been shown to exert adverse effects on many bodily systems. The pro-inflammatory cytokines IL-6 and TNF-α, together with interleukin-1 are the most important mediators of the so-called sickness response, which describes a series of depressionlike symptoms that occur during peripheral immune system activation. Frequently reported cognitive and behavioral symptoms of the sickness response are fatigue, pain, cognitive disturbances, depressed mood, and sleep disorders (Watkins et al 1995). Pro-inflammatory cytokines are further involved in mediating detrimental somatic processes, such as osteoporosis (Manolagas 1998; Papanicolaou et al 1998) and atherosclerosis (McCarty 1999; Straub et al 1998). Inappropriately high levels of IL-6 may trigger a constellation of changes that could contribute to the process of aging (Ershler et al 1994). IL-6 has also been reported to be associated with the development of disabilities and even mortality in elderly people (Ferrucci et al 1999).

Interestingly, epidemiologic studies have consistently found that persons with PTSD described more physical symptoms and mood changes than people without PTSD, suggesting links between trauma or PTSD and various somatic symptoms (Engel et al 2000). Andreski et al (1998) reported cardiovascular, pulmonary, and other somatization symptoms such as pain to be more common in PTSD patients compared with healthy subjects and patients suffering from other psychiatric disorders. Furthermore, Vietnam veterans with current PTSD have been shown to have a significantly higher risk for coronary heart disease (Boscarino and Chang 1999a; Falger et al 1992). Solomon proposed that the psychological distress caused by PTSD increases vulnerability to somatic problems (Solomon and Mikulincer 1987). Such symptoms may result from extremely stressful experiences in home countries (Castillo et al 1995). Ford et al (2001) suggested that physical symptoms were common after many severe or recurrent traumatic stressors. The biological link between traumatic experiences, PTSD, and physical symptoms is incompletely understood at present; however, the data reported here, together with current knowledge of the effects of pro-inflammatory cytokines, could lead to the conclusion that increased levels of pro-inflammatory cytokines may mediate these somatic symptoms. This interpretation would be in line with the concept of allostatic load, in which the constellation that we report here for PTSD patients serves as an example of a "lack of response." Increased levels of catecholamines, together with low levels of cortisol, are proposed to promote negative health consequences by disinhibition of other mediators that normally are downregulated by GCs—in this case, the secretion of pro-inflammatory cytokines (McEwen and Stellar 1993; Munck et al 1984).

As we show here, the decreased levels of free cortisol are partly counterregulated by an increased sensitivity of pro-inflammatory cytokine production to the suppressive effects of GCs; however, the increased cytokine-producing capacity of peripheral monocytes reported here suggests that the compensatory upregulation of GC sensitivity may be insufficient to protect the body from negative health outcomes. These results should be interpreted in the context of some limitations. First, the control group was not completely composed of Bosnian war refugees. Although we did not find any differences between German and

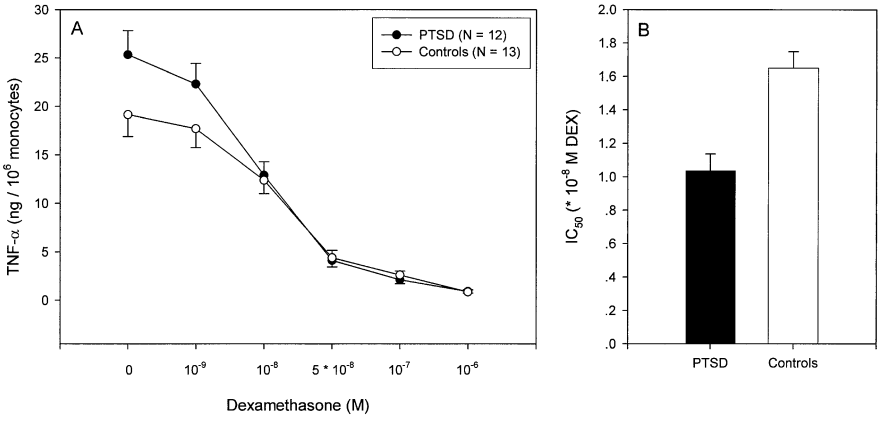

Figure 4. (A) Dexamethasone (DEX) inhibition of lipopolysaccharide-stimulated tumor necrosis factor-alpha (TNF-alpha) production and **(B)** inhibitory concentration 50 (IC_{50}) as an index for glucocorticoid sensitivity of stimulated TNF-α production in patients with posttraumatic stress disorder (PTSD) and in control subjects.

Bosnian control subjects, it cannot be completely excluded that the differences we found are associated with ethnicity. Furthermore, the diagnostic procedure used here (i.e., the use of a nonstandardized semistructured interview) is a clear limitation. Methodological limitations also include the fact that instructions did not explicitly prohibit tobacco use during the night of urine sampling and that self-reported abstinence from alcohol consumption was not controlled, for example, by measuring p450 enzymes. The urinary norepinephrine data could be confounded by dietary differences between patients that have not been controlled.

Also, it is obvious that the currently employed method of ex vivo stimulation of cytokine production and inhibition by GCs does not necessarily reflect in vivo changes in the organism; additionally, it should be noted that GC sensitivity is rather specific to target tissues; the findings reported here cannot be generalized, for example, to feedback sensitivity of the HPA axis. It is therefore a task for future studies to investigate whether the increased sensitivity reported here could be also found in nonimmunologic target tissues and whether this phenomenon can be reversed by successful therapeutic treatment.

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