Immune and Endocrine Function in Burnout Syndrome

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Objective: Burnout is a stress-induced work-related syndrome. It is associated with a higher incidence of infections possibly pointing to a compromised immune system. In the present study, endocrine and ex vivo immune function of severe cases of burnout were investigated. **Methods:** Endocrine and immune variables were compared in 56 persons with burnout and 38 healthy control subjects. Cortisol after awakening, after a low-dose dexamethasone, and dehydroepiandrosterone-sulphate (DHEAS) were analyzed from saliva. Peripheral blood was analyzed for T, B, and NK cell number and in vitro mitogen-induced pro- and antiinflammatory cytokine release. The capacity of dexamethasone to regulate cytokine release was compared between the groups. **Results:** The burnout group showed an increased production of the antiinflammatory cytokine interleukin-10 (IL-10) by monocytes after lipopolysaccharide stimulation. No differences were observed in IL-10 release induced by the T-cell mitogen PHA nor in the proinflammatory cytokines gamma interferon and tumor necrosis factor alpha. The capacity of dexamethasone to regulate cytokine release did not differ between the groups. The number of peripheral blood T cells, B cells, or NK cells was not different either. The burnout group showed higher DHEAS levels but no difference in cortisol levels after awakening or after dexamethasone intake in comparison to controls. **Conclusion:** Production of the antiinflammatory cytokine IL-10 by monocytes was increased in individuals with burnout syndrome. It seems unlikely that glucocorticoids or changes in glucocorticoid receptor function play a role in this higher IL-10 production. **Key words:** burnout, cytokines, IL-10, monocyte, glucocorticoids, DHEAS.

AUC = area under the curve; **BMI** = body mass index; **CAR** = cortisol awakening response; **CIS20R** = checklist individual strength; **CFS** = chronic fatigue syndrome; **DHEAS** = dehydroepiandrosterone-sulphate; **DST** = dexamethasone suppression test; **IFN-γ** = gamma interferon; **IL** = interleukin; **LPS** = lipopolysaccharide; **MBI-GS** = Maslach Burnout Inventory General Survey; **NK-cell** = natural killer cell; **PHA**=phytohemagglutinin; **SCL90** = symptom checklist; **TNF-α** = tumor necrosis factor alpha.

INTRODUCTION

Burnout is an adverse work-related syndrome characterized by persistent exhaustion, a cynical work attitude, and feelings of reduced competence (1). Additionally, people experiencing burnout report tension headaches, an inability to relax, gastrointestinal problems, muscle aches, disrupted sleep, concentration and memory problems, and depressive symptoms (2,3). There is considerable overlap of these symptoms in related syndromes like chronic fatigue syndrome (CFS) (4) and vital exhaustion (5). The core component of all of these syndromes is exhaustion, but the three components that characterize burnout are by definition work stress-related (2). Moreover, CFS is additionally defined by symptoms reflecting pain (4), whereas pain is not a central component of burnout. The vital exhaustion questionnaire score is used as a risk-factor for cardiovascular disease (5). Persons experiencing burnout report depressive symptoms, but the constructs of depression and burnout are not synonymous (2,6). In The Netherlands, a stable 9% of the working population report emotional exhaustion in the clinical burnout range. Emotional exhaustion predicts sickness absence, and over one third of the long-term sickness compensation claims are the result of psychological complaints (www.statline.nl).

Burnout is associated with an increased incidence of self-reported illnesses such as common cold, flu-like illness, and gastroenteritis (7). The core symptom of burnout, exhaustion, was shown to be the strongest predictor of infections, although the other aspects of burnout, cynicism (or depersonalization) and reduced competence (or personal accomplishment), also contributed to the prediction of infections. Whether this increased risk of common infections in persons with burnout is the result of dysregulation of immune function is the topic of the present study.

It is now widely accepted that chronic stress can lead to downregulation of immune function, which may impair an effective immune response to infectious challenges and thereby increase the risk of infectious diseases (8). Because burnout is the result of chronic work-related stress, it may thus be associated with decreased immune functioning as well. This study focuses on severe cases of burnout seeking psychological treatment for their complaints.

Few studies have examined immune function in relation to burnout symptoms. Nakamura et al. reported an association of a higher score on the burnout subscale depersonalization with lower natural killer cell activity (9). Bargellini et al. found that low personal accomplishment was associated with reduced numbers of total lymphocytes, T cells (CD3+), including the subsets of T cells CD4+ (T helper/inducer cells), and T suppressor/cytotoxic cells (CD8+) (10). Taken together, these studies demonstrate a reduced lymphocyte number and activity in relation to burnout, although it is remarkable that no relation was found with the core symptom of burnout, i.e., exhaustion. All of these studies, however, focused on individuals with burnout complaints in a working population who can be considered relatively healthy. To date, no studies on immune function in burnout have examined more severe cases of individuals with burnout.

In the present study, in addition to immune cell numbers, the capacity to produce cytokines by immune cells after in

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vitro stimulation was determined. Cytokines are soluble communicators between components of the immune system and the brain. They are involved in stress-induced behavioral and cognitive changes known as "sickness behavior" that resemble the side symptoms of burnout (11). Cytokines can be divided into proinflammatory cytokines such as interleukin (IL)-2, tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN)-γ and antiinflammatory cytokines like IL-4 and IL-10. T helper (Th) cells are categorized on the basis of their pro- or antiinflammatory cytokine production into T helper type 1 (Th1) and T helper type 2 (Th2) cells, respectively (12). Monocytes produce both proinflammatory and antiinflammatory cytokines. A disturbed balance between pro- and antiinflammatory cytokines has been found to be related to several infectious, autoimmune/inflammatory, and allergic diseases (12). Several stressors have been associated with a shift in cytokine production toward an antiinflammatory pattern with glucocorticoids and/or catecholamines as the proposed mediators of this shift (12). Because persons with burnout report more infections (7), which may be the result of an inadequate response to invading pathogens, one could hypothesize a shift toward antiinflammatory cytokine production to exist in those with burnout.

Some earlier studies have investigated cytokine release in persons with burnout and in related syndromes like CFS and vital exhaustion. Gaab et al. observed a positive correlation between reported fatigue and stimulated monocyte release of IL-6 and TNF-α in a group of patients diagnosed with CFS (13). Visser et al. observed higher IL-10 production by monocytes in patients with CFS (14). Considering exhaustion as the core component of burnout, we hypothesized to find higher antiinflammatory cytokine production by monocytes and T cells in persons with severe burnout.

The glucocorticoid cortisol and its synthetic analog dexamethasone are potent modulators of the immune system with immunosuppressive effects (12). We recently showed that there are no changes in salivary cortisol levels after awakening, during the day, or after the dexamethasone suppression test (DST) in a clinical burnout group compared with a healthy control group (15). Irrespective of whether the negative findings could be replicated in the present sample, we considered it worthwhile to measure cortisol parameters again to investigate their potential role in the immune function. The DST is an indication for glucocorticoid receptor sensitivity and subsequent negative feedback functioning of the hypothalamic–pituitary–adrenal axis (16). The steroid dehydroepiandrosterone-sulfate (DHEAS) also shows an immunomodulatory function but opposite from cortisol (17). Low DHEAS levels seem to be associated with poorer health (18), although Grossi et al. and Moch et al. found no differences in serum or plasma DHEAS in participants with burnout (19,20). A shift in the cortisol/DHEA(S) ratio toward cortisol has been associated with mood disorders and perceived stress (21).

The immune-modulating effect of cortisol not only depends on circulating cortisol levels, but also on receptor sensitivity of immune cells for glucocorticoids. Immune cells express glucocorticoid receptors, and in vitro exposure of peripheral blood lymphocytes to dexamethasone results in immunosuppression. Wirtz et al. found that more dexamethasone was required to suppress IL-6 production by monocytes in vitally exhausted men, pointing to a decrease in monocyte glucocorticoid receptor sensitivity (22). Visser et al. observed an increased sensitivity to dexamethasone in patients with CFS (23), whereas Kavelaars et al. found a significant reduction in the maximal effect of dexamethasone on phytohemagglutin (PHA) -induced T cell proliferation (24). In the present study, we investigated the inhibiting effect of increasing dexamethasone concentrations on PHA-stimulated IFN-γ and IL-10 cytokine release in persons with burnout as well as the dexamethasone effect on lipopolysaccharide (LPS) -stimulated TNF-α and IL-10 production by monocytes. In line with the studies in CFS, we expected to find an altered glucocorticoid sensitivity of T cells and monocytes in persons with burnout.

In summary, the goal of the present study is to identify potential changes in immune and endocrine function in burnout individuals, focusing on lymphocyte subset numbers, cortisol parameters, DHEAS, pro- and antiinflammatory cytokines, and the sensitivity of the immune cells to glucocorticoids.

METHODS

Participants

The burnout group consisted of 56 participants (25 men and 31 women), mean age 43.0 years (standard deviation [SD] = 9.3, range = 27–65 years). The control group included 38 participants (19 men and 19 women), mean age 44.8 years (SD = 8.6, range = 30–60 years). Seven persons were excluded from blood sampling as a result of medication use (n = 5) or refrained from having their blood sample taken (n = 2). There was no difference in age, gender, body mass index (BMI), or complaints in these persons compared with the rest of the burnout group.

Enrollment of the burnout group took place through different healthcare institutions, where the participants experiencing burnout had applied for treatment. In addition, information about the research project was posted on several burnout-related web sites. Interested participants received a screening questionnaire by mail or e-mail. Selection of the participants was based on cutoff scores indicating clinical burnout as reported by the Dutch version of the Maslach Burnout Inventory General Survey (MBI-GS) (exhaustion $\geq$2.20 and depersonalization $\geq$2.00 or competence $\leq$3.66) (25), severe fatigue (checklist individual strength; CIS20R) $\geq$76 (26), and exclusion of symptom check list (SCL90) scores within the psychopathological range (total score $>$214) (27). After selection, 52 participants with burnout received a clinical diagnostic interview by a qualified psychologist; details are described in more detail elsewhere (15). According to the diagnostic interview, 45 persons (87%) were diagnosed with the International Classification of Diseases, 10th Revision criteria for "work-related neurasthenia" and the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria for "undifferentiated somatoform disorder" (3,28). Participants with burnout had to be at the initial stage of treatment for their complaints and to report full or partial sickness absence. Medication use for asthma, rheumatoid arthritis, or diabetes and use of antidepressants were exclusion factors. Control participants were selected through a local newspaper advertisement and coworkers of the researchers. The data collection period of blood and saliva sampling was between August 2003 and December 2004. The participants in this study are a fresh selected sample; there is no overlap in participants between this study and our previously described studies (15,29). All participants gave written informed consent. Approval was obtained by the medical ethical committee of the University Medical Center Utrecht.

Questionnaires

The participants filled out questionnaires on demographic data, duration of complaints, stressful life events in the past 3 months (yes/no), and work status. Factors with a potential influence on cortisol such as smoking, menstrual cycle, the use of oral contraceptives, and medication were registered (30–32). In addition to the burnout inventory, several questionnaires were included that focused on complaints such as fatigue, poor sleep quality, depressive symptoms, and general psychopathology. The burnout symptoms exhaustion, cynicism, and feelings of reduced competence were measured with the Dutch version of the MBI-GS, 15-item version (33). Fatigue was assessed with the 20-item version of the Dutch fatigue scale CIS20R (34). Sleep quality of the past month was assessed with the Dutch State and Trait sleep assessment scale, 14-item version (GSKS (35)). Level of depressive symptoms was assessed with the Dutch version of the CES-D, 20 items (36). Finally, the Dutch version of the symptom checklist SCL90 was used as an indication for general psychopathology (27). All questionnaires are well-validated Dutch versions that have shown reasonable to good reliability.

Endocrine Measures

Saliva was collected at home for cortisol and DHEAS assessment. Samples were kept in the refrigerator and on return they were stored at −20°C. Saliva for cortisol analysis was sampled by a Salivette with a cotton roll (Sarstedt, Etten-Leur, The Netherlands) on 3 consecutive weekdays at 0, 15, and 30 minutes after awakening. The second evening, participants were instructed to take an oral dose of 0.5 mg dexamethasone. One person in the burnout group and three persons in the control group refrained from dexamethasone intake.

On the first 2 days of cortisol sampling, participants were instructed to collect an extra saliva sample at 30 minutes after awakening for DHEAS analysis by passive drool rather than by a cotton role (37). The samples were analyzed using a chemiluminescence assay (LIA) as described elsewhere (www.ibl-hamburg.com).

Immune Variables

Blood Collection Procedure

Participants were instructed to take a light meal on the morning of blood sampling and refrain from coffee, chocolate, and fruit intake. Blood was collected in 10-mL heparinized Vacu-tubes between 9:00 AM and 1:00 PM at the University Medical Center. The fatigue questionnaire (CIS20R) and the sleep quality scale (GSKS) were filled out. Questions on flu-like symptoms and common cold at the moment and in the past week, medication intake, and menstrual cycle date were reported.

Leukocyte Subpopulations

Circulating numbers of monocytes, T cell subsets, B cells, and natural killer (NK) cells were assessed in heparinized whole blood using dual-color fluorescence analysis with a Becton Dickinson Calibur flow cytometer. Whole blood was stained using monoclonal antibodies labeled with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) to quantify $CD14^+$ (monocytes), $CD3^+$ (total T), $CD4^+$ (T helper), $CD8^+$ (suppressor/cytotoxic-T), $CD19^+$ (B), $CD16^+CD56^+$ (NK), and $CD3^+/CD16^+CD56^+$ (NK-T) cells. Absolute numbers of cells were calculated from a total leukocyte blood count.

Cytokine Production

Whole blood diluted 1:10 with RPMI-1641 (Gibco, Grand Island, NY), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine was stimulated with the T cell mitogen PHA (Remel Europe Ltd., final concentration 25 μg/mL) at 37°C/5% CO_2 in 96-well round-bottom plates in the presence of 0, 1, 3, 10, 20, 50, 100, and 300 nmol/L dexamethasone. For IL-10 and IFN-γ determination, supernatants were collected after 72 hours of culture. Cytokine production was measured in culture supernatants using standard enzyme-linked immunosorbent assay (ELISA) kits (CLB, Amsterdam, The Netherlands).

To stimulate monocyte cytokine production, 1:10 diluted whole blood (with RPMI-1641 supplemented with antibiotics) was stimulated with LPS (*Escherichia coli* 0127: B8, Sigma, final concentration 2 ng/mL) at 37°C/5% CO_2 in 96-well flat-bottom plates. The effect of dexamethasone on cytokine production was assessed in the presence of the following dexamethasone concentrations: 0, 1, 3, 10, 20, 50, 100, and 300 nmol/L. Supernatants were collected after 24 hours of culture and IL-10 and TNF-α cytokine levels were measured using standard ELISA kits (CLB).

Statistical Analysis

The demographics and questionnaire scores of the burnout and the control group were compared using χ^2 test and one-way analysis of variance (ANOVA). One-way ANOVA was used to test for differences in endocrine and immune parameters. Positively skewed variables of the immune and endocrine data set were log transformed for the analysis, although the untransformed values are presented. A repeated-measures analysis was applied for analysis of the cortisol awakening response (CAR) with time after awakening as the within factor and group as the between factor. The cortisol (in nanomoles per liter) and DHEAS (in nanograms per milliliter) measurements on day 1 were significantly correlated with the measurements at day 2 (DHEAS: $r = 0.236$, $p = .029$, cortisol: 0 minutes; $r = 0.347$, $p = .001$, 15 minutes; $r = 0.553$, $p < .001$, 30 minutes; $r = 0.494$, $p < .001$). The data of the 2 days were pooled for further analysis. The CAR was recalculated into two area under the curve (AUC) measures: the AUC level, the amount of cortisol after awakening, and the AUC slope, the shape of the curve after awakening (38). Exclusion of persons with a negative slope (AUC slope <0; burnout group 16%, control group 18%), which is considered to be an indication of noncompliance (39), did not affect the results. The cortisol/DHEAS ratio was calculated by dividing the cortisol AUC level by the DHEAS level. The IFN-γ/ IL-10 ratio was calculated using the PHA-induced cytokine levels. Dexamethasone-inhibited cytokine production curves were analyzed using repeated-measures analysis with "dexamethasone concentration" as the within-subject factor and "group" as the between factor. Greenhouse-Geisser correction was applied. Logistic transformation of the positively skewed data set did not lead to different outcomes in the repeated-measures analysis; therefore, the nontransformed data set was used. Effect sizes are reported as partial eta squared (η_p^2) for the repeated-measures analysis and eta squared (η^2) for the one-way ANOVAs, 0.01 = small, 0.06 = moderate, and 0.14 = large (40). Eta squared is the proportion of the total variance that is attributed to an effect. An alpha level of 0.05 was used for all statistical tests.

RESULTS

Table 1 shows that there were no differences in gender, age, BMI, smoking, or oral contraceptive use between the burnout and the control group. The groups did not differ in reported common cold or flu-like symptoms, menstrual cycle phase during blood sampling, or the occurrence of a stressful life event in the past 3 months (data not shown). The control group reported more weekly strenuous exercise, more alcohol intake (data not shown), and a higher percentage of women in menopause (Table 1). From these potential confounders, only menopause showed an association with the outcome variables. The menopause group showed a lower number of monocytes and granulocytes. However, controlling for menopause status did not affect the results and therefore the noncorrected results are reported.

The burnout participants reported partial or complete sickness absence, the mean complaint duration at the intake of the study was 31.6 months (SD = 30.6), and 46% reported to have had work-related complaints before. The moment of cortisol sampling was on average 2 weeks apart from the blood sampling, but there was no difference between the burnout (mean = 0.47 months, SD = 1.3) and the control group (mean = 0.54 months, SD = 0.34) in this time interval ($F_{1,86} = 0.17$, $p = .68$).

TABLE 1. Demographic Variables and Complaints

	Burnout	Control	Test Value
Gender (male)	25 (45%)	19 (50%)	χ^2=0.261
Age, mean (SD)	43.0 (9.3)	44.8 (8.6)	F=0.95
Body mass index, mean (SD)	24.4 (4.0)	24.5 (3.2)	F=0.001
Smoker	13 (23%)	5 (13%)	χ^2=1.48
Women			
Contraceptive use	6 (19%)	7 (37%)	χ^2=1.87
Menopausal	3 (10%)	6 (32%)	χ^2=3.83[b]
Sickness absence			
Not	5 (9%)	38 (100%)	χ^2=75.65[c]
Partial	26 (46%)	—	
Fully	25 (45%)		
Medication use			
None	32 (57%)	27 (71%)	χ^2=1.87
Beta-blockers[a]	4 (7%)	0	
Benzodiazepines[a]	6 (11%)	0	
Hypertensive	2 (4%)	2 (5%)	
Antihistamine	2 (4%)	0	
Nonsteroidal antiinflammatory drugs	0	1 (3%)	
Other	10 (18%)	8 (21%)	

Burnout $n = 56$, control $n = 38$.
Data given as number (percentage) unless otherwise noted.
[a] In the blood sampling, two participants who used beta-blockers and one person who used benzodiazepines were excluded.
[b] $p < .05$; [c] $p < .001$.

Table 2 shows the questionnaire scores of the burnout and the control group. The burnout group reported more fatigue, depressive symptoms, lower sleep quality, and scored higher on general psychopathology compared with the control group. Fatigue and sleep quality were filled out twice, once during the intake and once more on the day of blood sampling. The reported complaints and the main physiological outcome variables were unrelated within the groups.

Endocrine Parameters

Table 3 shows the mean scores of the endocrine variables. Repeated-measures analyses showed a significant increase in cortisol after awakening (time: $F_{2,146} = 37$, $p < .001$, partial eta squared (η_{p^2}) = 0.29), but no difference between the groups (groups: $F_{1,92} = 0.16$, $p = .69$, $\eta_{p^2} < 0.01$) nor a difference between the groups in the increase (slope) after awakening (group × time: $F_{2,146} = 0.58$, $p = .52$, $\eta_{p^2} < 0.01$). Cortisol levels after dexamethasone showed a small, but significant increase after awakening (time: $F_{2,137} = 3.7$, $p = .04$, $\eta_{p^2} = 0.04$), but no difference between the groups (groups: $F_{1,87} = 1.3$, $p = .26$, $\eta_{p^2} = 0.02$) nor a group difference in the increase after awakening (group × time: $F_{2,137} = 0.54$, $p = .54$, $\eta_{p^2} < 0.01$). The burnout group showed significantly higher DHEAS levels compared with the control group, but the cortisol/DHEAS ratio

TABLE 2. Test Variables in the Burnout and the Control Group

	Burnout ($n = 56$)	Control ($n = 38$)	F[a]
Burnout (Maslach Burnout Inventory General Survey)			
Exhaustion	4.76 (0.97)	1.17 (0.53)	430.10
Depersonalization	3.51 (1.50)	1.08 (0.64)	88.69
Competence	3.55 (1.32)	4.70 (0.84)	22.79
Fatigue (checklist individual strength)			
At inclusion	106.48 (16.26)	43.18 (13.10)	399.42
At blood sampling	93.63 (19.34)	38.61 (12.12)	235.52
Sleep quality (Dutch State and Trait sleep assessment scale, 14-item version)			
At inclusion	7.05 (3.85)	2.31 (2.83)	42.15
At blood sampling	6.21 (3.84)	2.29 (2.78)	28.20
Depressive symptoms (Center for Epidemiologic Studies–Depression)	25.58 (9.19)	4.32 (3.63)	182.92
General psychopathology (symptom checklist)	181.36 (32.75)	104.20 (10.29)	197.13

Data given as means (standard deviation).
[a] All F values are significant at the $p < .001$ level and $\eta^2 > 0.20$.

TABLE 3. Endocrine Parameters in the Burnout and the Control Group

	Burnout (n = 56)	Control (n = 38)	F	η^2
Cortisol				
0 min	15.26 (5.69)	15.53 (6.19)	0.05	0.001
15 min	19.70 (7.51)	18.68 (7.37)	0.43	0.005
30 min	21.18 (8.11)	20.40 (6.9)	0.23	0.003
Area under the curve level	37.92 (13.04)	36.65 (12.29)	0.23	0.002
Area under the curve slope	7.41 (8.97)	5.58 (9.16)	0.93	0.010
Cortisol after dexamethasone suppression test[a]				
0 min[b]	1.10 (1.38)	1.40 (1.60)	1.53	0.017
15 min[b]	1.01 (1.59)	1.72 (2.87)	2.65	0.029
30 min[b]	1.53 (2.44)	1.98 (3.10)	0.67	0.007
DHEAS (ng/mL)[b]	3.49 (2.77)	2.30 (1.83)	5.50[c]	0.056
Cortisol/DHEAS ratio[b]	19.30 (19.95)	26.98 (22.05)	2.95	0.032

Cortisol (nmol/L), DHEAS (ng/mL).
Data given as mean (standard deviation).
[a] Dexamethasone suppression test in burnout group n = 55, control group n = 35.
[b] Analysis of variance on log-transformed data.
[c] $p < .05$.
DHEAS = dehydroepiandrosterone-sulphate.

was not different. The difference in DHEAS level remained after introducing gender, age, and BMI as covariates in the analysis.

Leukocyte Cell Count

The number of leukocytes was not different between the burnout (10.0×10^6 cells/mL blood, SD = 2.9) and the control group (9.6×10^6 cells/mL blood, SD = 2.8) ($F_{1,85} = 0.43$, $p = .51$, $\eta^2 < 0.01$). Moreover, there was no difference in mean erythrocyte sedimentation rate between the burnout group (7.0 mm/hour, SD = 6.1) and the control group (7.7 mm/hour, SD = 7.4) ($F_{1,84} = 0.194$, $p = .67$, $\eta^2 < 0.01$). There were no differences between the groups in mean granulocyte, monocyte or lymphocyte number, nor in numbers of B cells (CD19+), NK cells (CD16+ 56+), T cells (CD3+), T helper/inducer cells (CD4+), T suppressor/cytotoxic cells (CD8+), or CD4+/CD8+ratio (data not shown). There was a significant difference in a subset of T cells expressing CD16/ 56 determinants (NK-T cells) (log NK-T; $F_{1,86} = 4.13$, $p = .045$, $\eta^2 = 0.046$), i.e., the burnout group (mean = 0.08, SD = 0.07) had fewer NK-T cells compared with the control group (mean = 0.14, SD = 0.20).

Phytohemagglutinin and Lipopolysaccharide-Induced Cytokine Secretion

Table 4 shows the mean cytokine levels in the supernatants of PHA-stimulated T cells and LPS-stimulated monocytes. Interestingly, the burnout group shows a significantly higher LPS-induced IL-10 production by monocytes compared with the control group with a medium to large effect size. No other differences were observed between the groups in monocyte TNF-α, T cell IFN-γ, T cell IL-10 release, nor in the T cell IFN-γ/ IL-10 ratio. *Within* the burnout group, the IL-10 levels showed no dose–response relationship with any of the psychological complaints.

Regulation of Cytokine Secretion by Dexamethasone

Figure 1 shows the dexamethasone inhibition of cytokine release in PHA-stimulated T cell cultures. Increasing dexamethasone concentrations significantly inhibited IFN-γ (Fig. 1A) and IL-10 production (Fig. 1B) (dexamethasone concentration: $F_{2,567} = 42, 53$, $p < .001$, $\eta_{p^2} = 0.34, 0.40$, respectively), but no group differences were apparent.

TABLE 4. Mitogen-Induced Cytokine Release in the Burnout and the Control Group

	Burnout	Control	F	η^2
T cell				
Gamma interferon (×1000)[a]	16.3 (28.3)	13.0 (13.4)	0.21	0.003
Interleukin-10[a]	298.3 (286.2)	308.4 (344.7)	0.11	0.001
Gamma interferon/interleukin-10 ratio[a]	69.3 (128.9)	50.2 (42.7)	0.07	0.001
Monocyte				
Tumor necrosis factor alpha	881.5 (343.9)	855.0 (253.9)	0.15	0.002
Interleukin-10[a]	52.5 (38.0)	33.6 (24.9)	9.01[b]	0.100

Burnout n = 49, control n = 38.
Nontransformed means (pg/mL) and (standard deviation).
[a] Analysis of variance on log-transformed data.
[b] $p < .01$.

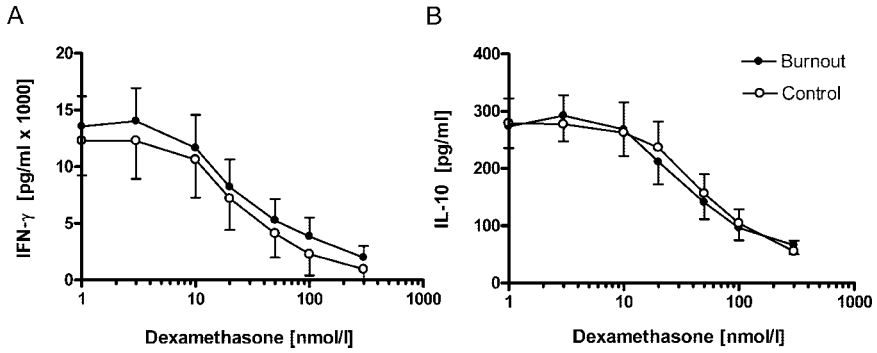

Figure 1. Dexamethasone inhibition of T cell cytokine release. Whole blood samples were cultured in the presence of T cell mitogen phytohemagglutinin and increasing dexamethasone concentrations. Graphs show the mean and standard error of the burnout (●) and the control group (○) for (**A**) gamma interferon and (**B**) interleukin-10. Basal cytokine release in the absence of dexamethasone is shown in Table 4.

Figure 2 shows the dexamethasone effect on LPS-stimulated monocytes. Dexamethasone significantly inhibited TNF-α release (Fig. 2A; dexamethasone concentration: $F_{2,567} = 403$, $p < .001$, $\eta_{p^2} = 0.83$), but the inhibitory effect was not different between the groups. LPS-induced IL-10 release (Fig. 2B) increased at low dexamethasone concentrations with a peak at 50 nmol/L and decreased at higher dexamethasone levels (dexamethasone concentration: $F_{7,574} = 9.9$, $p < .001$, $\eta_{p^2} = 0.11$). The overall IL-10 level was significantly higher in the burnout group compared with the control group (groups: $F_{1,82} = 6.0$, $p = .017$, $\eta_{p^2} = 0.07$). The significant interaction of the groups with the dexamethasone concentration (group × dexamethasone: $F_{2,152} = 3.2$, $p = .05$, $\eta_{p^2} = 0.04$) became nonsignificant after controlling for the initial IL-10 level.

DISCUSSION

This is the first study that focuses on possible immune changes in severe cases of burnout on sick leave. Despite the wide range of parameters investigated, no evident changes in most endocrine, receptor, and immune functioning were observed. There was an increased release of the antiinflammatory cytokine IL-10 by monocytes (but not T cells) in the burnout group compared with the control group. The burnout group showed a higher level of DHEAS but no difference in the cortisol/DHEAS ratio.

Results of studies in CFS, a syndrome with analogous symptoms, may serve as a reference for our results. In the review of Lyall et al., the results of several studies on immune function in CFS are summarized. Their main conclusion was that there are no clear differences between patients with CFS and normal control subjects with respect to T, B, or NK cell numbers and function or cytokine levels (41). A similar review by Patarca concluded that there is a predominance of T cells producing proinflammatory cytokines in CFS, but that no other clear interpretable patterns emerge (42). A credit to the present study is that only few studies differentiated between the antiinflammatory IL-10 release by T cells and monocytes. The observed higher IL-10 release by monocytes is in concordance with Visser et al. who reported higher LPS-induced IL-10 levels in CFS (14). In contrast, Gupta et al. did not observe a difference in LPS-activated monocyte IL-10 release in six patients with CFS compared with six healthy control subjects, although there was a decrease in PHA-induced IL-10 and spontaneously produced IL-10 (43).

What could be the significance of a higher IL-10 release in burnout? As mentioned before, persons with burnout report more common cold, flu-like infections, and gastroenteritis (7). Vital exhaustion is associated with an increased pathogen burden (i.e., response to antibodies for herpes simplex virus, varicella zoster virus, Epstein-Barr virus, and cytomegalovi-

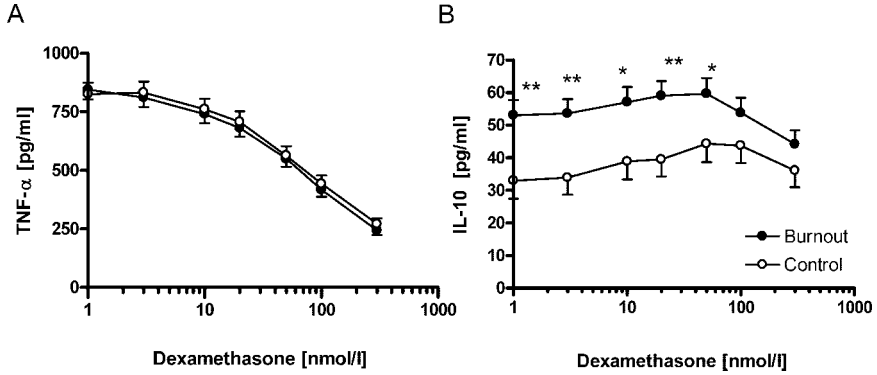

Figure 2. Dexamethasone regulation of monocyte cytokine release. Whole blood samples were incubated in the presence of lipopolysaccharide to stimulate monocyte cytokine release and increasing dexamethasone concentrations. Graphs show the mean and standard error of the burnout (●) and the control group (○) for (**A**) tumor necrosis factor alpha and (**B**) interleukin-10. Basal cytokine release in the absence of dexamethasone is shown in Table 4. *$p < .05$, **$p < .01$.

rus) and with higher serum levels of IL-10 (44). Moreover, psychosocial stress, a longer duration of stressful life events, and enduring problems related to work are associated with increased risk of developing a cold after exposure to the common cold virus (45). The observed immune dysregulations in our study show peculiar similarity with a study on a viral common cold infection (46). The rhinovirus is the most important common cold virus (47). In vitro exposure of immune cells to rhinovirus causes an inhibition of T cell proliferation mediated by a virus-dependent increase in monocyte IL-10 release. The amounts of IL-10 and the number of monocytes producing this cytokine on stimulation with the rhinovirus were comparable with those seen after LPS stimulation (46). These data suggest that the higher IL-10 release in burnout like we observed might be causally related to a common cold virus infection. IL-10 exerts its antiinflammatory effect through inhibition of macrophage activation, T cell proliferation, and proinflammatory cytokine production, i.e., inhibition of the antiviral cytokine IFN-γ (12), thus promoting the increased viral load. However, the burnout and the control group in our study did not differ in the reported cold or flu-like symptoms at the time of blood sampling or the week before. The duration of common cold symptoms is typically 1 week, whereas 25% of the persons infected with a common cold do not show signs of the infection (47). Our finding may point to a subclinical viral infection, which may have contributed to the exhaustion symptoms. Glaser et al. underlined the importance of latent virus reactivation or subclinical viral infections in CFS. Different types of psychological stressors can reactivate latent Epstein-Barr virus, which in turn upregulate IL-10 production in monocytes (48).The possibility that the changes in immune parameters in burnout as observed are the result of subclinical infections is worth further investigation.

The burnout and the control group were not different in the effect of dexamethasone on the cytokine release or in the level of cortisol in saliva. The effect of dexamethasone is an indicator of glucocorticoid receptor functioning. These data imply that glucocorticoids and the glucocorticoid receptor are not critically involved in the observed changes in IL-10 release by stimulated monocytes in the burnout group. The absence of a difference in salivary cortisol levels between the groups is in accordance with the findings in our previous study (15). The burnout group showed a higher level of DHEAS. The observed higher DHEAS level contradicts studies suggesting a role of lower DHEAS in deteriorated health (18,49). So far studies in burnout found no changes in plasma DHEAS levels (19,20). Although DHEAS is considered to be stable (49,50), the correlation between the DHEAS levels on the 2 days in our study was significant but small. DHEAS has been associated with increased IL-10 production (51), and DHEA, the nonsulfatized form of DHEAS, has been found to reduce susceptibility to viral, bacterial, and protozoan infections (17). However, DHEA and DHEAS can also have immunostimulatory effects (17). Therefore, the relevance of the increased DHEAS level in burnout for immune function remains to be determined.

In conclusion, we showed that burnout is associated with changes in monocyte antiinflammatory immune function, which may point to some role of (subclinical) infection. A possible role for DHEA(S) remains to be elucidated.

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REFERENCES

1. Maslach C, Schaufeli WB, Leiter MP. Job burnout. Annu Rev Psychol 2001;52:397–422.
2. Schaufeli WB, Enzmann D. The Burnout Companion to Study and Practice: A Critical Analysis. London: Taylor & Francis, 1998.
3. ICD-10; International Statistical Classification of Diseases and Related Health Problems. Geneva: World Health Organization, 1994.
4. Reeves WC, Lloyd A, Vernon SD, Klimas N, Jason LA, Bleijenberg G, Evengard B, White PD, Nisenbaum R, Unger ER. Identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution. BMC Health Services Research 2003;3:25.
5. Appels A, Mulder P. Fatigue and heart disease. The association between 'vital exhaustion' and past, present and future coronary heart disease. J Psychosom Res 1989;33:727–38.
6. Shirom A. Reflections on the study of burnout. Work and Stress 2005; 19:263–70.
7. Mohren DCL, Swaen GMH, Kant I, van Amelsvoort LGPM, Borm PJA, Galama JMD. Common infections and the role of burnout in a Dutch working population. J Psychosom Res 2003;55:201–8.
8. Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Psychoneuroimmunology and psychosomatic medicine: back to the future. Psychosom Med 2002;64:15–28.
9. Nakamura H, Nagase H, Yoshida M, Ogino K. Natural killer (NK) cell activity and NK cell subsets in workers with a tendency of burnout. J Psychosom Res 1999;46:569–78.
10. Bargellini A, Barbieri A, Rovesti S, Vivoli R, Roncaglia R, Borella P. Relation between immune variables and burnout in a sample of physicians. Occup Environ Med 2000;57:453–7.
11. Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol Rev 1998;105:83–107.
12. Elenkov IJ, Chrousos GP. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. Ann N Y Acad Sci 2002;966: 290–303.
13. Gaab J, Rohleder N, Heitz V, Engert V, Schad T, Schurmeyer TH, Ehlert U. Stress-induced changes in LPS-induced pro-inflammatory cytokine production in chronic fatigue syndrome. Psychoneuroendocrinology 2005;30:188–98.
14. Visser J, Graffelman W, Blauw B, Haspels I, Lentjes E, de Kloet ER, Nagelkerken L. LPS-induced IL-10 production in whole blood cultures from chronic fatigue syndrome patients is increased but supersensitive to inhibition by dexamethasone. J Neuroimmunol 2001;119:343–9.
15. Mommersteeg PMC, Heijnen CJ, Verbraak MJPM, van Doornen LJP. Clinical burnout is not reflected in the cortisol awakening response, the day-curve or the response to a low-dose dexamethasone suppression test. Psychoneuroendocrinology 2006;31:216–25.
16. Cole MA, Kim PJ, Kalman BA, Spencer RL. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 2000;25:151–67.
17. Chen CC, Parker CR Jr. Adrenal androgens and the immune system. Seminars in reproductive medicine 2004;22:369–77.
18. Wolf OT, Kirschbaum C. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. Brain Res Brain Res Rev 1999;30:264–88.
19. Moch SL, Panz VR, Joffe BI, Havlik I, Moch JD. Longitudinal changes in pituitary–adrenal hormones in South African women with burnout. Endocrine 2003;21:267–72.
20. Grossi G, Perski A, Evengard B, Blomkvist V, Orth-Gomer K. Physiological correlates of burnout among women. J Psychosom Res 2003;55: 309–16.
21. Young AH, Gallagher P, Porter RJ. Elevation of the cortisol–dehydroepiandrosterone ratio in drug-free depressed patients. Am J Psychiatry 2002;159:1237–9.

22. Wirtz PH, von Kanel R, Schnorpfeil P, Ehlert U, Frey K, Fischer JE. Reduced glucocorticoid sensitivity of monocyte interleukin-6 production in male industrial employees who are vitally exhausted. Psychosom Med 2003;65:672–8.
23. Visser J, Blauw B, Hinloopen B, Brommer E, de Kloet ER, Kluft C, Nagelkerken L. CD4 T lymphocytes from patients with chronic fatigue syndrome have decreased interferon-gamma production and increased sensitivity to dexamethasone. J Infect Dis 1998;177:451–4.
24. Kavelaars A, Kuis W, Knook L, Sinnema G, Heijnen CJ. Disturbed neuroendocrine–immune interactions in chronic fatigue syndrome. J Clin Endocrinol Metab 2000;85:692–6.
25. Schaufeli W-B, Bakker A-B, Hoogduin K, Schaap C, Kladler A. On the clinical validity of the Maslach Burnout Inventory and the Burnout Measure. Psychology and Health 2001;16:565–82.
26. Bultmann U, de Vries M, Beurskens AJHM, Bleijenberg G, Vercoulen JHMM, Kant I. Measurement of prolonged fatigue in the working population: determination of a cutoff point for the checklist individual strength. J Occup Health Psychol 2000;5:411–6.
27. Arrindel WA, Ettema JHM. Dimensionele structuur, betrouwbaarheid en validiteit van de Nederlandse bewerking van de Symptom Checklist (SCL-90) [Dimensional structure, reliability and validity of the Dutch translation of the Symptom Checklist (SCL-90)]. Nederlands Tijdschrift voor de Psychologie, vol 36. Lisse, The Netherlands: Swets & Zeitlinger, 1981:77–108.
28. Diagnostic and Statistical Manual of Mental Disorders. Washington DC: American Psychiatric Association, 1994.
29. Mommersteeg PMC, Keijsers GPJ, Heijnen CJ, Verbraak MJPM, van Doornen LJP. Cortisol deviations in people with burnout before and after psychotherapy; a pilot study. Health Psychol 2006;25:243–8.
30. Canals J, Teresa Colomina M, Domingo JL, Domenech E. Influence of smoking and drinking habits on salivary cortisol levels. Personality and Individual Differences 1997;23:593–9.
31. Kirschbaum C, Kudielka BM, Gaab J, Schommer N, Hellhammer DH. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus–pituitary–adrenal axis. Psychosom Med 1999;61:154–62.
32. Pruessner JC, Wolf OT, Hellhammer DH, Buske Kirschbaum A, von Auer K, Jobst S, Kaspers F, Kirschbaum C. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci 1997;61:2539–49.
33. Schaufeli WB, Van Dierendonck D. UBOS, Utrechtse Burnout Schaal [UBOS, the Dutch Translation of the Maslach Burnout Inventory (MBI)]. Lisse, The Netherlands: Swets & Zeitlinger BV, 2000.
34. Vercoulen JHMM, Alberts M, Bleijenberg Gvd. The checklist individual strength. Gedragstherapie 1999;32:131–6.
35. Meijman TF, Thunnissen MJ, de Vries-Griever AG. The after-effects of a prolonged period of day-sleep on subjective sleep quality. Work and Stress 1990;4:65–70.
36. Bouma J, Ranchor AV, Sanderman R, Sonderen Ev. Het meten van symptomen van depressie met de CES-D [Measuring Symptoms of Depression With the CES-D]. Groningen: Noordelijk Centrum voor Gezondheidsvraagstukken, 1995:1–24.
37. Shirtcliff EA, Granger DA, Schwartz E, Curran MJ. Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology 2001;26:165–73.
38. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 2003;28:916–31.
39. Kudielka BM, Broderick JE, Kirschbaum C. Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. Psychosom Med 2003;65:313–9.
40. Cohen J. Statistical Power Analysis for the Behavioral Sciences. Hillsdale, NJ: Lawrence Erlbaum Associates, 1988.
41. Lyall M, Peakman M, Wessely S. A systematic review and critical evaluation of the immunology of chronic fatigue syndrome. J Psychosom Res 2003;55:79–90.
42. Patarca R. Cytokines and chronic fatigue syndrome. Ann N Y Acad Sci 2001;933:185–200.
43. Gupta S, Aggarwal S, See D, Starr A. Cytokine production by adherent and non-adherent mononuclear cells in chronic fatigue syndrome. J Psychiatr Res 1997;31:149–56.
44. van der Ven A, van Diest R, Hamulyak K, Maes M, Bruggeman C, Appels A. Herpes viruses, cytokines, and altered hemostasis in vital exhaustion. Psychosom Med 2003;65:194–200.
45. Cohen S, Frank E, Doyle WJ, Skoner DP, Rabin BS, Gwaltney JM Jr. Types of stressors that increase susceptibility to the common cold in healthy adults. Health Psychol 1998;17:214–23.
46. Stockl J, Vetr H, Majdic O, Zlabinger G, Kuechler E, Knapp W. Human major group rhinoviruses downmodulate the accessory function of monocytes by inducing IL-10. J Clin Invest 1999;104:957–65.
47. Gwaltney J, Jack M. Clinical significance and pathogenesis of viral respiratory infections. Am J Med 2002;112:13–8.
48. Glaser R, Padgett DA, Litsky ML, Baiocchi RA, Yang EV, Chen M, Yeh P-E, Klimas NG, Marshall GD, Whiteside T. Stress-associated changes in the steady-state expression of latent Epstein-Barr virus: implications for chronic fatigue syndrome and cancer. Brain Behav Immun 2005;19:91–103.
49. Kroboth P, Salek F, Pittenger A, Fabian T, Frye R. DHEA and DHEA-S. A review. J Clin Pharmacol 1999;39:327–48.
50. Assies J, Visser I, Nicolson NA, Eggelte TA, Wekking EM, Huyser J, Lieverse R, Schene AH. Elevated salivary dehydroepiandrosterone-sulfate but normal cortisol levels in medicated depressed patients: preliminary findings. Psychiatry Res 2004;128:117–22.
51. Cheng G-F, Tseng J. Regulation of murine interleukin-10 production by dehydroepiandrosterone. J Interferon Cytokine Res 2000;20:471–8.