ORIGINAL ARTICLE

Cytokine-Associated Emotional and Cognitive Disturbances in Humans

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Background: Infectious, autoimmune, and neurodegenerative diseases are associated with profound psychological disturbances. Studies in animals clearly demonstrate that cytokines mediate illness-associated behavioral changes. However, the mechanisms underlying the respective psychological alterations in humans have not been established yet. Therefore, we investigated the effects of low-dose endotoxemia, a well-established and safe model of host-defense activation, on emotional, cognitive, immunological, and endocrine parameters.

Methods: In a double-blind, crossover study, 20 healthy male volunteers completed psychological questionnaires and neuropsychological tests 1, 3, and 9 hours after intravenous injection of *Salmonella abortus equi* endotoxin (0.8 ng/kg) or saline in 2 experimental sessions. Blood samples were collected hourly, and rectal temperature and heart rate were monitored continuously.

Results: Endotoxin had no effects on physical sickness symptoms, blood pressure, or heart rate. Endotoxin caused a mild increase in rectal temperature (0.5°C), and increased the circulating levels of tumor necrosis factor α (TNF-α), soluble TNF receptors, interleukin (IL)-6, IL-1 receptor antagonist, and cortisol. After endotoxin administration, the subjects showed a transient significant increase in the levels of anxiety (effect size [ES] = 0.55) and depressed mood (ES = 0.66). Verbal and nonverbal memory functions were significantly decreased (ES = 0.55 to 0.64). Significant positive correlations were found between cytokine secretion and endotoxin-induced anxiety ($r = 0.49$ to $r = 0.60$), depressed mood ($r = 0.40$ to $r = 0.75$), and decreases in memory performance ($r = 0.46$ to $r = 0.68$).

Conclusions: In humans, a mild stimulation of the primary host defense has negative effects on emotional and memory functions, which are probably caused by cytokine release. Hence, cytokines represent a novel target for neuropsychopharmacological research.

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INFECTIOUS DISEASES are associated with profound behavioral disturbances. These are collectively termed *sickness behavior* (SB) and include malaise, fatigue, depression, anorexia, hyposomnia or hypersomnia, decreased physical and social activities, and cognitive disturbances.[1-3] Several lines of evidence suggest that inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin (IL)-1, and IL-6, meditate these disturbances. In animals, SB can be induced by administration of cytokines, and antagonists or synthesis blockers of cytokines abolish SB in response to various immune challenges.[2-5] Studies in humans indicate that administration of cytokines (particularly interferons and IL-2) produces behavioral alterations similar to SB in animals, as well as depressive symptoms and impairments of memory, attention, and executive functions.[6-10] However, these studies are limited because of 2 major reasons: (1) In general, effects of cytokines have been studied in severely ill patients, and thus may add to or interfere with different preexisting medical and psychological conditions. (2) The high doses that have been administered induce prominent physical sickness symptoms that by themselves are likely to compromise cognitive performance and the patients' emotional condition. Therefore, it is desirable to investigate the emotional and cognitive effects of experimental immunostimulation in healthy subjects.

The only established experimental model for assessing the acute host response to infection in humans is the administration of endotoxin (lipopolysaccharide). Purified endotoxin is not infectious, but provides a potent acute stimulus of primary host responses.[11] In

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SUBJECTS AND METHODS

SUBJECTS

The study was approved by an independent ethics committee. Twenty male subjects (mean age, 23.7±3.3 years; range, 19-30 years; mean years of education, 15.4±2.9; range, 13-23; and mean body weight, 73.1±9.6 kg; range, 52-90 kg) participated in the study. Subjects were recruited by advertisements presented on the advertising boards of the universities in Munich. Before detailed screening, the subjects were informed about the study design, including information about the immunological and endocrine effects of endotoxin, and the extent and time course of symptoms to be expected. Afterward, each subject went through a complete physical and psychiatric assessment. The physical examination included electroencephalogram, electrocardiogram, blood sedimentation rate, complete blood cell count, liver enzymes, electrolytes, urinalysis, quantitative urine screening for cannabinoids, amphetamines, opioids, benzodiazepines, barbiturates, cocaine metabolites, and phenylcyclidine, as well as hepatitis B virus test. Moreover, an interview was conducted by an experienced psychiatrist to evaluate the presence and the history of any Axis I psychiatric disorder according to *DSM-IV*.[18] Exclusion criteria were as follows: (1) presence of any medical illness; (2) presence of any clinically significant abnormality in blood and urine tests (including evidence for recent drug use), in the electroencephalogram, electrocardiogram, and neuropsychological tests assessing memory, attention, and executive functions (Figure Recall, Digit Span Forward, and Trail Making Test [TMT A and B], respectively); (3) history of allergies, autoimmune, liver, and other severe or chronic diseases; (4) presence or history of any Axis I psychiatric disorder; (5) history of seizures; (6) actual or recent (during the last 2 weeks) intake of any kind of prescription or nonprescription drug; and (7) shift work or time zone shifts (>3 hours) during the last 6 weeks. Subjects who successfully passed the screening procedure were considered eligible to participate in the experiment and enrolled after written informed consent had been obtained.

PROCEDURE

The study was conducted in a clinical research unit using a balanced, randomized, double-blind, crossover design. All technical equipment, including the blood sampling device, was housed in a room adjacent to the sound-shielded experimental room. Every subject passed 2 testing sessions, separated by 10 days. Subjects spent the night before each experimental session in the research unit. Upon their first arrival in the evening, a battery of neuropsychological and emotional tests, assessing memory, learning, attention, executive functions, and mood was given for adaptation and to control for practice effects.[19] Different versions of these tests were used in the experimental testing sessions. In the next morning, an intravenous cannula was inserted into an antecubital forearm vein for intermittent blood sampling and drug injection. At 9 AM each subject was injected intravenously with endotoxin (0.8 ng of *S abortus equi* endotoxin per kilogram of body weight) in one session and with the same volume of 0.9% saline solution in the other session. *Salmonella abortus equi* endotoxin had been prepared for use in humans and was available as a sterile solution free of proteins and nucleic acids (for more details, see Galanos et al[20]). This preparation has proven to be safe in various studies of other groups (for review, see Burrell[12]) and in studies at the Max Planck Institute of Psychiatry, including more than 80 subjects since 1991.[15-17] The order of injections was balanced, so that half of the subjects received the saline injection and half received the endotoxin injection first. No significant differences were found between the groups defined by the treatment order in age, years of education, or body weight. The experimenter and the subject were blind with respect to the group assignment. For safety reasons, a physician was aware of the subject's group assignment, but he did not take part in the testing procedures. Yet, this physician was also permanently on call during every experimental session. During each session, subjects were tested 3 times, at 1 to 2, 3 to 4, and 9 to 10 hours postinjection (see shaded areas over **Figure 1**), using different versions of the neuropsychological tests every time. Blood was collected at baseline, and at hourly intervals up to 10 hours postinjection. Rectal temperature was measured continuously using a thermistor probe. Heart rate was monitored continuously and blood pressure hourly. At the end of each experimental day, subjects underwent a thorough physical examination before discharge from the clinical research unit.

NEUROPSYCHOLOGICAL ASSESSMENT

The neuropsychological test battery assessed verbal and nonverbal memory, learning, attention, and executive functions. Six parallel test versions were used, so each subject was administered a different version on each of the 3 testing periods in each of the 2 testing sessions (see the "Procedure" subsection of the "Subjects and Methods" section). The order in which the different test versions were

experimental animals, endotoxin rapidly induces the production and secretion of cytokines, and elicits SB symptoms, which can be blocked by antagonists, synthesis blockers, or antibodies to proinflammatory cytokines.[3,4] In humans, endotoxin administration was found to induce a "flu-like" syndrome characterized by fever, malaise, and increased production and secretion of cytokines (particularly TNF-α, IL-6, and IL-1 receptor antagonist) and cortisol. Studies using purified endotoxin preparations for use in humans from *Escherichia coli* or from *Salmonella abortus equi* have shown that low doses are safe and well tolerated.[12-17] Extent and duration of endotoxin-induced host responses are dose-dependent. For example, low amounts of *S abortus equi* endotoxin (0.8 ng/kg body weight) administered to healthy volunteers in the morning have been shown to induce clear-cut increases in the circulating levels of cytokines and cortisol, but no physical sickness symptoms.[15-17]

Hence, we used this dose and preparation of endotoxin to test the hypotheses that endotoxin negatively affects mood, the level of anxiety, memory, attention, and executive functions in healthy volunteers. Moreover, we explored whether these effects are quantitatively related to the secretion of cytokines or cortisol.

administered was counterbalanced across subjects to avoid any nonrandom version-dependent bias.[19] The individual tests were presented in a fixed order in all 6 versions.

Memory and learning were assessed using 3 tests. Story Recall[21]: subjects are requested to repeat a 25-item story from memory immediately, and 30 minutes after presentation. Figure Recall[22,23]: subjects are instructed to copy an 18-item figure, and to reproduce it from memory 3 and 30 minutes later, and Word List Learning[24]: subjects are requested to immediately repeat a 15-word list. This procedure is repeated 5 consecutive times. For all memory tests the total number of correct verbatim recall is counted. The Ruff 2 and 7 cancellation test,[25] the Digit Span Forward,[26] and the Digit Symbol[26] tests were used to assess attention. The numbers of correct responses were counted. Attention was also assessed using a computerized Simple Reaction Time test,[27] in which subjects are instructed to press the space bar on a keyboard as soon as they see a digit, as well as the Continuous Performance Test,[27] in which subjects are instructed to press the space bar as soon as they see 2 identical digits one after the other. The mean response time in both tasks was measured. The colored TMT A and B[28] and the Word Fluency test[23,29] were used to assess executive functions. The time needed to complete the TMT A and B, and the sum of correct words produced for the 3 letters in the Word Fluency test were counted.

Emotional state was assessed during the middle of each testing period. Depressed mood was assessed using the Depression Adjectives Check List,[30] and anxiety was assessed using the State Anxiety Inventory.[31]

BEHAVIORAL ASSESSMENT

Physical sickness symptoms (headaches, muscle pain, shivering, nausea, breathing difficulties, and fatigue) were assessed at the end of each testing period, by a questionnaire using a 5-point Likert scale (0, no symptoms, to 4, very severe symptoms). Food and water consumption were measured at 0 to 4 (consumption of crackers), 4 to 5 (standardized lunch), and 5 to 10 hours (consumption of crackers).

PLASMA LEVELS OF CYTOKINES AND CORTISOL

Plasma blood was collected in tubes containing sodium ethylenediaminetetraacetic acid and aprotinin, and was immediately centrifuged, aliquoted, and frozen to –20°C. The plasma level of cortisol was determined by a radioimmunoassay, and the plasma levels of cytokines and soluble cytokine receptors were assessed by commercial enzyme-linked immunosorbent assays (for more details see Mullington et al[32]).

STATISTICAL ANALYSES

The main hypothesis concerning treatment effects on emotional status and neuropsychological performance was tested using repeated-measures analyses of variance. Analyses of variance were also used to examine the treatment effect on physical sickness symptoms, plasma levels of cytokines and cortisol, and body temperature. The level of significance was set at the critical value of $P=.05$ (2-tailed). Whenever significant treatment × time interactions were found, the simple effects were analyzed as suggested by Winer,[33] and Scheffe's adjustments were applied. Effect sizes (ES) were calculated using Cohen's formula.[34] To assess the associations between changes from the placebo to the endotoxin condition in physiological (cytokines and cortisol secretion) and psychological (emotional states and neuropsychological performance) parameters, Pearson correlation coefficients were calculated. In these correlations, the variable that represented the difference between endotoxin- and placebo-induced secretion of cytokines and cortisol was computed as the cumulative area under the response curve (the area between the endotoxin and placebo curves) for each cytokine and for cortisol. This variable was calculated for each time interval in which psychological assessment was conducted (ie, 0-2, 0-4, and 0-10 hours postinjection). In addition, partial correlations were calculated to estimate the independent associations between the physiological and psychological parameters. For example, the partial correlation between TNF-α secretion and the change in anxiety level is the correlation between the change in TNF-α levels and the change in anxiety level, after controlling for their mutual association with cortisol secretion.[35] Since the intercorrelations between the cytokines were very high ($r>0.8$), they were not entered simultaneously into the analyses.[36]

The assumption of normal distributions of the dependent variables was assessed.[36] No deviation from normality was evident for any of the dependent variables. Univariate outliers were assessed using z scores,[36] and multivariate outliers were assessed using the Mahalanobis distance.[36] No outliers were found with either method. To adjust for any nonhomogeneity of covariance for the within-subject effects, we used P values that were adjusted using the Huynh-Feldt (H-F) method.[35] Analyses were carried out using SPSS 9.

RESULTS

PHYSIOLOGICAL EFFECTS OF ENDOTOXIN

Endotoxin induced a significant increase in heart rate during all testing periods ($F_{1,18}=4.5$, $P=.04$) (data not shown). Endotoxin did not produce any significant effects on systolic or diastolic blood pressure levels, or on the subjective rating of physical sickness symptoms (data not shown). Food, but not water consumption was significantly reduced in the first 4 hours postinjection (46.6±6.6 vs 64.9±8.0 g, in the endotoxin and saline conditions, respectively; $t_{19}=3.1$; $P=.006$). Endotoxin induced a significant increase in plasma levels of TNF-α, soluble TNF receptor p55, soluble TNF receptor p75, and IL-6, which peaked during the first testing period and monotonically declined thereafter (Figure 1A-C). These effects were reflected by significant treatment × time interactions ($F_{7,126}=43.8$, 26.7, 14.6, and 18.5, respectively, all $P<.001$, by the H-F method). Plasma levels of IL-1 receptor antagonist started to rise during the first testing period, peaked during the second, and were still elevated at the last period ($F_{7,126}=90.6$, $P<.001$, by

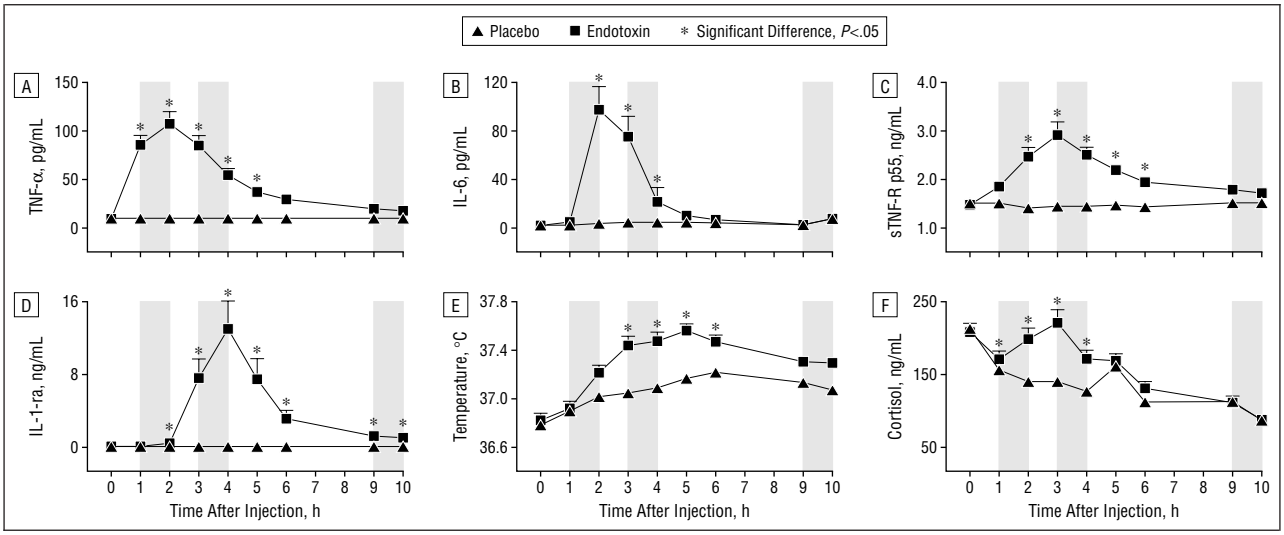

Figure 1. *Changes over time (mean±SEM) in the plasma levels of tumor necrosis factor α (TNF-α) (A); interleukin (IL)-6 (B); soluble TNF receptor p55 (a very similar pattern was obtained for soluble TNF receptor [sTNFr] p75) (C); IL-1 receptor antagonist (ra) (D); rectal temperature (E); and cortisol (F). All measures showed a significant treatment × time interaction (all values below P<.005) (N=20). Shaded areas indicate the times subject were tested postinjection.*

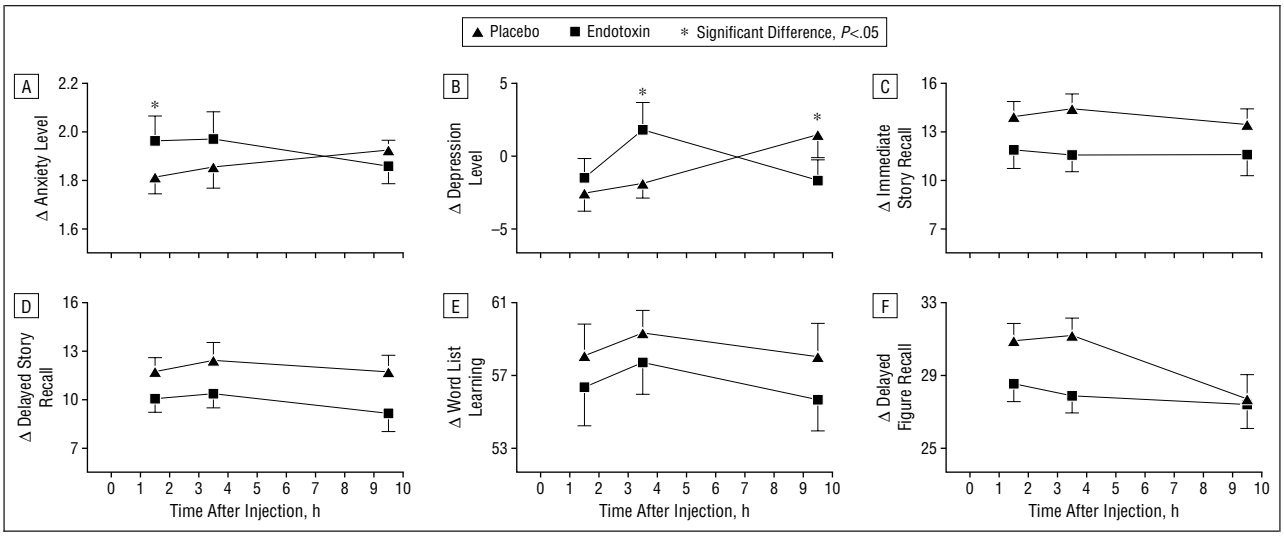

Figure 2. *Endotoxin-induced changes over time (mean±SEM) in emotional and memory parameters (N=20). The effects of endotoxin on anxiety levels (A), depressed mood (B), immediate Story Recall (C), delayed Story Recall (D), Word List Learning (E), and delayed Figure Recall (F) were measured at 1, 3, and 9 hours after either endotoxin or placebo injection.*

H-F method, for a treatment × time interaction) (Figure 1D). Endotoxin induced a significant increase in rectal temperature (reaching about 0.5°C), which started during the first testing period, and peaked during the second period (Figure 1E). This finding was reflected by a significant treatment × time interaction ($F_{7,126}=8.4$, $P<.001$, by H-F method). The endotoxin-induced elevation in cortisol levels began at the first session, peaked at the second, and returned to placebo levels at the last ($F_{7,126}=5.8$, $P<.001$, by H-F method, for a treatment × time interaction) (Figure 1F).

EMOTIONAL AND COGNITIVE EFFECTS OF ENDOTOXIN

A significant endotoxin-induced increase in anxiety level was observed at 1 to 2 hours postinjection, but not later on. This finding was reflected by a significant treatment × time interaction ($F_{2,36}=5.4$, $P=.009$, ES=0.55). Endotoxin produced a significant increase in depressed mood, which was evident at 3 to 4 hours, and was followed by relatively lower levels of depressed mood at 9 to 10 hours postinjection. This effect was reflected by a significant treatment by time interaction ($F_{2,36}=7.7$, $P=.003$, by H-F method; ES=0.66) (**Figure 2**).

Endotoxin did not cause any significant changes in measures of attention or executive functions (ie, the simple reaction time, Continuous Performance Test, Digit Cancellation, Digit Symbol, Digit Span, TMT A and B, or Word Fluency tests) during any testing session. In contrast, endotoxin administration produced a global decrease in memory functions, during all testing periods, reflected by decreased immediate recall of story items ($F_{1,18}=7.1$; $P=.01$; ES=0.62), reduced delayed Story Recall ($F_{1,18}=5.4$; $P=.03$; ES=0.55), a deficit in immediate and delayed recall of figure items ($F_{1,18}=8.8$, $P=.008$, ES=0.70; $F_{1,18}=7.5$,

P=.01, ES=0.64, respectively), and decreased performance in Word List Learning ($F_{1,18}$=6.9, P=.01, ES=0.61) (Figure 2). No order effects were evident in any of the emotional or cognitive measures.

All analyses of variance are based on a priori hypotheses regarding elevated negative mood and decreased cognitive performance, and therefore no correction for multiple comparisons was necessary.[36] However, it should be noted that even after Bonferroni corrections, all mood and memory tests (except the delayed recall of the logical memory) remained significant (P=.01).

Endotoxin-induced changes in anxiety level were significantly (P<.05) and positively correlated with the secretion of each cytokine in the first testing period (r=0.49 to r=0.60) (**Figure 3**). Endotoxin-induced changes in depressed mood were significantly (P<.01) and positively correlated with the secretion of each cytokine in the first and second testing periods (r=0.40 to r=0.75), but not in the last period (Figure 3). Endotoxin-induced impairments in immediate and delayed Story Recall were significantly (P<.01) and positively correlated with the secretion of every cytokine in the first testing period (r=0.55 to r=0.68), and endotoxin-induced impairments in immediate and delayed Figure Recall were significantly (P<.05) correlated with the secretion of every cytokine, in the first and second testing periods (r=0.46 to r=0.63), but not in the last period (Figure 3). The association between endotoxin-induced changes in Word List Learning and cytokine secretion was positive, but it did not reach significance. Endotoxin-induced changes in depressed mood (at 1 to 2 and 3 to 4 hours postinjection), and Figure Recall (at all testing periods) were also significantly (P<.05) and positively correlated with serum cortisol secretion (r=0.44 to r=0.74). In contrast, endotoxin-induced decrease in depressed mood at 9 to 10 hours postinjection was significantly (P<.05) and negatively correlated with serum cortisol secretion (r=–0.48).

Partial correlation analysis with each cytokine completely eliminated the correlation between cortisol secretion and both anxiety and memory impairments (**Table**). However, the partial correlations between depressed mood and cortisol secretion as well as between depressed mood and cytokine secretion were still significant, suggesting that cytokines and cortisol are independently associated with endotoxin-induced increase in depressed mood.

Partial correlation analysis was also employed to examine the relationships between the emotional and memory changes. After controlling for the association between cytokines and memory, no significant correlations were found between anxiety or depression levels and any of the memory functions.

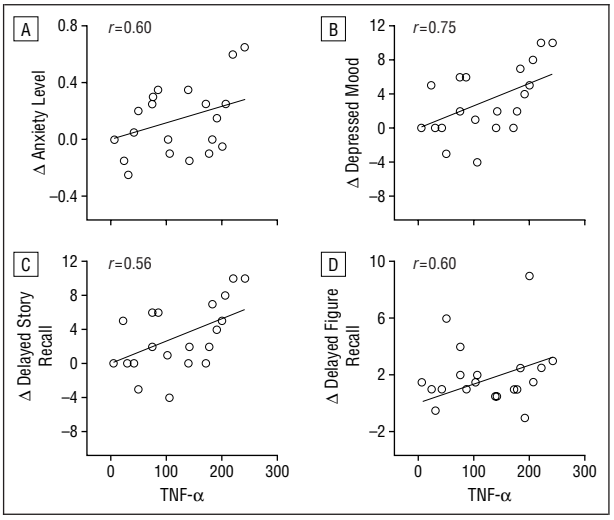

Figure 3. *Associations between cytokine secretion and psychological variables. Exemplified are the associations between tumor necrosis factor α (TNF-α) secretion and the changes (Δ) in anxiety in the first testing period (A), depressed mood in the second testing period (B), delayed Story Recall in the first testing period (C), and delayed Figure Recall in the first testing period (D). The associations between these psychological changes and the secretion of interleukin 6 and interleukin 1 receptor antagonist were very similar. The numbers at the top right of each graph represent the correlation coefficient between the change in psychological measures and TNF-α secretion (N=20).*

COMMENT

The results of this study demonstrate that experimental immune activation by endotoxin produces alterations in emotional states and decreased performance in memory tests. In this study, we used a dose of endotoxin that consistently stimulates cytokine production without inducing subjective feelings of illness. Thus, the endotoxin-induced increase in anxiety and depressed mood and the decrement in performance in both verbal and nonverbal memory cannot be attributed to a perceived illness-associated distress.

Endotoxin-induced changes in emotional parameters were found to have a complex time course, characterized by an early elevation in anxiety levels, followed by an increase in depressed mood, which later reversed into relatively lower levels of depressed mood. An elevation in anxiety and depression levels after immune challenges has been documented in both clinical and experimental settings.[9,37-41] Our findings extend these reports by demonstrating that endotoxin-induced increases in anxiety and depressed mood are strongly associated with the extent of cytokine secretion. Both anxiety and depression were also significantly associated with cortisol secretion. However, the time course of cortisol secretion, which began to increase when anxiety was already present, and the results of the partial correlation analysis, which demonstrated that the association between cortisol and anxiety is entirely mediated by the association between cytokines and anxiety, suggest that increased anxiety is produced through the actions of cytokines, rather than hypothalamic-pituitary-adrenal system activation. In contrast, the simultaneous elevation in cortisol secretion and depressed mood, and the independent associations between cytokines, cortisol, and depressed mood, suggest that both cytokine secretion and hypothalamic-pituitary-adrenal activation contribute to endotoxin-induced increase in depressed mood. This is consistent with previously suggested roles for both cytokines[41] and hypothalamic-pituitary-adrenal system activation (particularly corticotropin-releasing hormone secretion)[42] in depressive symptoms.

Inflammatory cytokines have also been implicated in cardiovascular disease.[43] Increasing knowledge about the effects of cytokines on mood might be very stimu-

Partial Correlations Between Endotoxin-Induced Cytokines and Cortisol Secretion and Changes in Psychological Variables*

	Testing Period	SAI	DACL	Immediate Story Recall	Delayed Story Recall	Immediate Figure Recall	Delayed Figure Recall
TNF-α†	1	0.49‡	0.59§	0.48‡	0.58§	0.35	0.46‡
Cortisol	1	0.08	0.48‡	0.03	0.22	0.16	0.18
TNF-α	2	0.31	0.26	0.40‡	0.41‡	0.36	0.48‡
Cortisol	2	−0.05	0.38‡	0.19	0.19	0.02	0.15
TNF-α	3	−0.22	0.02	0.24	0.29	0.02	−0.15
Cortisol	3	−0.37	−0.49‡	0.09	0.17	0.27	0.61§

*SAI indicates State-Trait Anxiety Inventory; DACL, Depression Adjectives Check List; and TNF-α, tumor necrosis factor α.

†Exemplified here are the associations between TNF-α secretion (area under the response curve) and the changes in anxiety (measured by the SAI), depressed mood (measured by the DACL), and immediate and delayed Story and Figure Recall, while controlling for the effects of cortisol. The partial correlations between these psychological changes and the secretion of interleukin 6 and interleukin 1 receptor antagonist were very similar.

‡P<.05.

§P<.01.

lating for the discussions about the association between depression and cardiovascular disease. This association is at present mainly discussed from the perspective of a presumed negative influence of depression on the cardiovascular system.[44] However, it is tempting to speculate that cytokines may mediate a negative influence of cardiovascular disease on mood.

Somewhat unexpectedly, depressed mood ratings were lower in the endotoxin condition compared with placebo, at 9 to 10 hours after injection. Similarly, depressed patients given a high dose of endotoxin in the evening "rapidly exhibited pronounced apathy," but reported improved mood 15 hours after endotoxin administration, when cytokine levels and fever subsided.[45] In that study, a high amount of endotoxin was administered, inducing chills and an increase in rectal temperature to about 39°C. Therefore, improved mood in the following morning might indicate psychological relief caused by the disappearance of physical sickness symptoms. However, in the present study subjects did not perceive major physical sickness symptoms. Therefore, the biphasic mood response to endotoxin might indicate that very low amounts of cytokines, which the subjects were exposed to at the end of the present experiment, might have a positive effect on mood, in contrast to the negative effect of higher concentrations reached rapidly after the injection of endotoxin. Interestingly, very low amounts of endotoxin (0.2 ng/kg) administered at night promote slow-wave sleep, whereas the same dose as administered in the present study induces a transient sleep disturbance.[32] Hence, it seems worthwhile to pursue the idea that inflammatory cytokines might influence some complex brain functions in opposite directions, depending on the amounts present in the brain. For this purpose, a study similar to the present one, but including various doses of endotoxin, would be useful.

The results of the present study indicate that experimental immune activation produces a global decrement in performance of declarative memory: both verbal and nonverbal, immediate and delayed memory functions were decreased. These findings are consistent with previous research in clinical populations, reporting that memory impairments are a common adverse effect of cytokine (especially interferon) therapy,[46] and viral (eg, influenza) infection.[1] The findings are also consistent with research in animals, which demonstrated that peripheral or central administration of either endotoxin or IL-1β produces decreased performance in various learning and memory paradigms.[47-50] Endotoxin-induced memory decrements in performance are relatively long-lasting, as they were evident even 10 hours after the injection, when all of the other responses to endotoxin (fever, anorexia, anxiety, and depressed mood) had already subsided. The decrease in memory functions was probably not secondary to the changes in either anxiety or depression, because memory was affected even after the mood effects had resolved, and because there were no correlations between the mood and cognitive changes, at each testing period.

The endotoxin-induced decrease in memory performance were selectively associated with cytokine secretion in the first 2 testing periods, and did not correlate with cortisol secretion, suggesting that these effects of endotoxin are not mediated by hypothalamic-pituitary-adrenal system activation. This notion is further supported by the finding that the decrease in memory performance was present before increases in cortisol levels. Although in previous studies stress and glucocorticoids were associated with decreased memory performance in humans,[51-59] the levels of cortisol in these studies were higher than those measured in the present experiment. Furthermore, a recent study[59] reported that after cortisol administration memory functions (as measured by the Story Recall test) were impaired. However, in that study the decrease in memory performance did not parallel the rise in plasma cortisol levels, and appeared only after 4 days of administration. Thus, whereas cortisol secretion does not seem to contribute to the decrease in memory performance that is associated with acute host defense activation, it may still be detrimental in more chronic conditions of immune activation.

The endotoxin-induced memory decrement, approximating 0.5- to 1.0-SD reductions from placebo level performance, is clinically relevant. For example, similar 1-SD changes in performance on the story recall test on the Wechsler Memory Scale would lower an individual's age-normalized classification by 1 level.[60] Although the endotoxin-induced increase in anxiety and depression also had a 0.5-SD magnitude, the absolute severity of negative mood did not reach clinical levels.

Our study has several limitations. First, the finding of a correlation between cytokine secretion and psychological measures does not provide conclusive evidence that

cytokines directly mediate the psychological changes. Second, because the levels of cytokines are highly correlated, it is difficult to draw any conclusion with respect to the specific role of each cytokine in mediating the observed psychological effects. Finally, in the present experiment endotoxin was given acutely, whereas most infectious conditions have a more chronic nature. In future studies, it will be necessary to gather direct evidence for a causative role of cytokines, using cytokine antagonists, such as soluble TNF receptors, which very recently have become available.[61] The use of such compounds should also clarify the role of specific cytokines in the psychological effects of endotoxin. Finally, other experimental models of immune activation and cytokine secretion should be used to demonstrate chronic effects.

The results of this study indicate that activation of the immune system by low doses of endotoxin can produce significant emotional and memory disturbances, which are positively correlated with endotoxin-induced cytokine secretion. Elevation of cytokine secretion, both in the periphery and within the brain, is associated not only with infectious diseases, but also with autoimmune diseases (eg, multiple sclerosis, rheumatoid arthritis), stroke, brain trauma, and neurodegenerative disease, such as Alzheimer disease.[62] Depression and anxiety are highly prevalent in all of these conditions.[41] Memory impairments are also commonly associated with infectious[1] and autoimmune diseases,[63] and are particularly evident in conditions that involve central inflammatory processes, including stroke and neurodegenerative diseases.[24] Obviously, in such conditions several pathophysiological mechanisms are involved in producing the emotional and the memory deficits, but the results of the present study suggest that at least some of the illness-induced behavioral pathology may be directly caused by cytokine secretion. This hypothesis has important implications for the development of new psychopharmacological approaches that should target the negative psychological effects of cytokines in various medical conditions.

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