Clinical Depression and Regulation of the Inflammatory Response During Acute Stress

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Objective: This study examined whether clinical depression is associated with a differential inflammatory response to an acute bout of psychological stress. **Methods:** A total of 72 women participated in the study; half met diagnostic criteria for clinical depression; the others had no history of psychiatric illness. The groups were matched with respect to age and ethnicity. All subjects were exposed to a 17-minute mock-job interview; blood was drawn to assess secretion and regulation of inflammatory molecules. **Results:** The stressor was associated with feelings of shame and anxiety, a mobilization of monocytes, neutrophils, and C-reactive protein into the circulation, and greater endotoxin-stimulated production of interluekin-6 and tumor necrosis factor-α by white blood cells in vitro. Depressed subjects began the session with greater sensitivity to the antiinflammatory properties of glucocorticoids than control subjects. Following exposure to the stressor protocol, however, sensitivity decreased among depressed subjects and increased among controls. This was manifest by disparities in interluekin-6 and tumor necrosis factor-α production in the presence of dexamethasone. **Conclusions:** These findings suggest that under acutely challenging conditions, depression is associated with greater resistance to molecules that normally terminate the inflammatory cascade. An impaired capacity to regulate inflammation could underlie some of the excess morbidity and mortality that has been associated with depression. **Key words:** depression, inflammation, acute stress, cortisol, cytokines, reactivity.

IL-6 = interleukin-6; **TNF-α** = tumor necrosis factor-α; **IC_{50}** = 50% inhibitory concentration; **LPS** = lipopolysaccharide.

INTRODUCTION

Depression is a risk factor for morbidity and mortality due to a variety of medical conditions (1–11). In an effort to identify the mechanisms responsible for this phenomenon, researchers have begun studying relationships between depression and inflammation, a process that is central to the development and progression of a number of diseases, including those in the cardiac, metabolic, rheumatologic, and autoimmune domains (12–17). Clinical depression is associated with marked increases in systemic inflammation, as evidenced by 40 to 50% elevations in circulating concentrations of C-reactive protein and interleukin-6 (IL-6 (18–21)). When their cells are stimulated in vitro with bacterial products such as lipolysaccharide, clinically depressed individuals also show an amplified inflammatory response, as manifest by higher levels of the cytokines IL-1β, IL-6, and tumor necrosis factor-α (TNF-α (22–25)). Finally, in community-dwelling nonpsychiatric populations, the presence of depressive symptoms is associated with elevations in circulating inflammatory molecules such as C-reactive protein and IL-6 (26–31).

Though this line of inquiry has yielded a number of important findings, it has focused solely on the *tonic* association between depression and inflammation, without exploring how these processes relate during acutely challenging situations. This is a potentially important oversight because disparities in the inflammatory response to stressors could help to explain some of the excess morbidity and mortality associated with depression. The goal of the current research was to begin examining this hypothesis in a cohort of healthy young adults who did not have a history of medical problems or treatments that might produce spurious associations among stressors, depression, and immunity. Thus, we enrolled a sample of young women who were either clinically depressed or without psychiatric illness and exposed them to a brief episode of acute stress. Blood was drawn repeatedly to monitor a variety of immune parameters, including the mobilization of leukocyte subsets, the expression of circulating inflammatory molecules, and white blood cells' capacity to produce inflammatory cytokines when exposed to bacterial products in vitro. We also assessed stress-related changes in white blood cells' sensitivity to dexamethasone, a synthetic version of the hormone cortisol that has potent anti-inflammatory properties (32). This was motivated by the hypothesis that depressed individuals may have diminished sensitivity to the anti-inflammatory properties of glucocorticoid hormones, perhaps as a result of this condition sometimes being associated with elevated resting levels of cortisol (33–35).

Based on previous research, we expected that the stressor would elicit marked increases in negative emotion and salivary cortisol, a mobilization of monocytes and neutrophils into the peripheral circulation, and enhanced white blood cell production of the inflammatory cytokines IL-6 and TNF-α (36). We also expected that the immune system's sensitivity to dexamethasone inhibition would decline following the stressor, as manifest by a reduction in this hormone's capacity to suppress production of IL-6 and TNF-α. This pattern has emerged in our studies of healthy adults (37–39), and it makes sense from an evolutionary perspective: in the context of a life-threatening situation, removing inhibitory constraints on the immune response would expedite the clearing of pathogens and the healing of wounds (36,40).

Perhaps most importantly, we expected the stressor to elicit different patterns of response in depressed and control subjects. Given that enhanced mood reactivity is often present in those with affective disorders, we expected depression to be associated with greater stress-related increases in negative

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emotion. We also expected depression to be associated with a *reduced* cortisol response to the stressor. This pattern of "blunting" has been documented in a number of studies (41,42), including a recent meta-analysis of the literature in this area (43). Because depression is not generally associated with abnormalities in leukocyte distribution (44), we did not expect to find differences in cell mobilization following the stressor. Finally, we predicted that depression would be associated with dysregulated cytokine production following the stressor. This would involve depression amplifying the stress-elicited boost in TNF-α and IL-6 production, and further dampening the stress-elicited decline in the capacity of dexamethasone to inhibit cytokine production.

METHODS

Subjects

A total of 72 women participated in the study; half of them met diagnostic criteria for clinical depression; the other half had no lifetime history of psychiatric illness. The groups were matched on a case-by-case basis with respect to age and ethnicity. All subjects were in good health, defined as having (a) no history of chronic medical illness, (b) no indications of acute infectious disease at study entry, as evidenced by self-report of symptoms and a normal complete blood count, and (c) no prescribed medication regimen in the past 6 months *including* antidepressants. We made an exception for women who were using oral contraceptives, because excluding them would have seriously jeopardized recruitment. Candidates were excluded if they were older than 55 years; had been pregnant in the past year; were menopausal, postmenopausal, or had irregular menstruation; were undernourished as evidenced by serum albumin $\leq$3.3 g/dl; or reported abusing illicit drugs. Our decision to focus the study on women, rather than both genders, was based on 2 primary considerations: the higher prevalence of depression in females and the desire to minimize extraneous variance that would arise in a mixed-gender sample.

Depressed subjects were recruited through advertisements in local newspapers seeking people who were "feeling down and depressed, losing interest in enjoyable activities, or having trouble eating, sleeping, or concentrating." To qualify for the study, depressed subjects had to meet diagnostic criteria for a current Major Depressive Episode (N = 32) or Minor Depressive Episode (N = 4) according to DSM-IV (45). Diagnoses were made by trained interviewers using the Depression Interview and Structured Hamilton (46). Subjects with comorbid psychotic, eating, alcohol, substance (other than Nicotine Dependence), or anxiety disorders (other than Generalized Anxiety Disorder) were excluded using modules from the Diagnostic Interview Schedule (47) and the Primary Care Evaluation of Mental Disorders (48). Control subjects were also recruited through newspaper advertisements; these postings sought "medically healthy adults for a study of mood and health." To qualify for the study, control subjects had to match a depressed subject in terms of age and ethnicity and had to have a lifetime history free of medical and psychiatric illness, as documented in structured psychiatric interviews using the Depression Interview and Structured Hamilton and modules from the Diagnostic Interview Schedule and the Primary Care Evaluation of Mental Disorders. They also needed to score $<$5 on the 10-item Center for Epidemiologic Studies Depression Scale (49).

We received a total of 404 responses to advertisements. Many of these respondents were excluded after a telephone-screening interview revealed a history of medical or psychiatric illness or a standing medication regimen that rendered them ineligible to participate (N = 150; 37.1%). Another large cohort of respondents was excluded during telephone interviews after reporting too much distress to qualify for the control group (Center for Epidemiologic Studies Depression Scale $\geq$5) but not enough functional impairment to be likely to meet diagnostic criteria for clinical depression (N = 70; 17.3%). Other respondents were excluded because they did have an appropriate demographic match (N = 13, 3.2%), were no longer interested in the study after the telephone interview, or were unable to find a mutually agreeable time to participate (N = 63, 15.6%). The remaining 108 subjects (26.8%) were scheduled for and attended a baseline laboratory session. Interviews conducted during this session revealed that 36 of these subjects (8.9%) had medical illnesses or psychiatric disorders that were not detected during the telephone contact. They were paid $20 for their time and were excluded from participation. The remaining 72 subjects comprise the sample for this report.

Procedures

Subjects visited the laboratory on 2 occasions. During the first session they provided written informed consent and underwent structured psychiatric and medical history interviews to determine eligibility. Qualified subjects then collected 3 days worth of daily diary data as they went about their normal routines (data not shown).

One week later subjects returned to the laboratory. They arrived between 8:00 AM and 10:00 AM after having observed an overnight fasting period during which food, alcohol, and caffeine were avoided. Subjects were then seated in a comfortable chair and, after a 10-minute adaptation period, had 3 blood pressure readings collected at 2-minute intervals (Dinamap Pro 100; Critikon Corp, Tampa, FL). Data on height, weight, and waist/hip circumference were then collected using standard methodology.

Subjects were then reseated. After a butterfly needle had been placed in the antecubital region of the nondominant arm, subjects relaxed quietly for 30 minutes as they acclimated to the presence of the needle. Baseline saliva and blood samples were collected at the end of this period. A 17-minute acute stressor was then administered (see below). Blood and saliva samples were again collected after the stressor. Subjects spent the next 30 minutes sitting quietly by themselves. During this time, saliva was collected every 10 minutes, and blood was drawn at the end of the period to assess recovery. On completing the protocol, subjects were paid $150. These procedures were approved by the Institutional Review Board of Washington University.

Stressor Protocol

The stressor consisted of a 17-minute mock job interview that was modeled after the Trier Social Stress Test (50). Subjects were led into a room occupied by 2 confederates sitting behind a table. One of the confederates introduced himself as the chair of the evaluation committee and instructed subjects to prepare a brief speech outlining their qualifications for a fictional position at a local company. For the next 5 minutes subjects were allowed to prepare their speeches, as members of the evaluation committee sat quietly watching them do so. The committee chair then switched on a tape recorder, asked subjects to discard any notes they had made, and instructed them to begin speaking about their qualifications. After 3 minutes of speaking had ensued, committee members stopped the subject and spent the next 4 minutes asking follow-up questions. These queries focused on subjects' prior work experiences, personal strengths and weaknesses, and long-term goals. Next, the committee chair announced that he needed to evaluate candidates' ability to work with others. To do so, he instructed subjects to solve a puzzle in collaboration with another committee member. The puzzle required subjects to navigate a toy car out of a crowded parking lot (Rush Hour–Traffic Jam; ThinkFun Corp; Alexandria, VA). Subjects were not allowed to either touch the car or point at it, but had to verbally direct the confederate's actions. The team was given 5 minutes to solve the puzzle. Only 5 of the 72 subjects (6.9%) succeeded within this timeframe. Those who did were immediately given a more difficult puzzle to solve. Subjects were aware from the start of the session that the job interview was fictional and had no implications for their employment situation.

To evaluate whether the mock job interview elicited negative emotions, subjects were asked to complete a brief questionnaire before and after it was administered. It consisted of 12 mood adjectives drawn from the Profile of Mood States and the Differential Emotions Scale (51). Using a 5-point intensity scale that ranged from "not at all" to "extremely," subjects indicated how well each word described their usual feelings (pretask) or their feeling during the job interview (post-task). The adjectives formed 4 three-item subscales reflecting states of anger (α = 0.83), anxiety (α = 0.89), shame (α = 0.84), and sadness (α = 0.90).

Outcome Measures

We assessed a number of biological outcomes before, during, and after the subjects completed the stressor protocol.

Salivary Cortisol

Saliva was collected by having subjects chew on a cotton dental roll for 60 seconds (Salivette; Sarstedt Corporation; Rommelsdorf, Germany). The saturated dental roll was then placed in a plastic centrifuge tube and spun for 5 minutes at 750 *g*. After the supernatant had been aspirated, it was frozen at −70°C until the study was completed. Cortisol was later measured in duplicate with a commercially available chemiluminescent technique (IBL-Hamburg; Hamburg, Germany). This assay has a sensitivity of 0.16 ng/ml and intra- and inter-assay coefficients of variation <12%.

Leukocyte Subsets

A complete blood count with differential was performed on blood samples collected during the laboratory session. It yielded circulating numbers of white blood cells, neutrophils, monocytes, and lymphocytes. Counts were performed immediately after each laboratory session, using an automated 5-part hematology analyzer from Beckman-Coulter (Fullerton, CA).

Circulating Inflammatory Molecules

To quantify circulating concentrations of IL-6, TNF-α and C-reactive protein, we drew 10 ml of blood into serum-separator tubes (Vacutainers; Becton-Dickinson, Franklin Lakes, NJ). After the blood had been centrifuged for 25 minutes at 1000 *g*, the serum was aspirated, divided into aliquots, and frozen at −70°C until the end of the study. Concentrations of IL-6 and TNF-α were measured in duplicate using commercially available, high-sensitivity ELISAs (R&D Systems; Minneapolis, MN). The sensitivity of these assays is 0.7 and 0.12 pg/ml, respectively, and the intra-assay coefficients of variation are 4.6 and 5.9%. C-reactive protein was measured using high-sensitivity immunoassay on a BN-100 nephelometer (Dade-Behring, Deerfield, IL). This technique has a lower detection threshold of 0.175 mg/L and intra- and inter-assay coefficients of variation of <3%.

Regulation of Inflammatory Cytokines

To examine stress-related changes in the regulation of inflammatory cytokines, we exposed white blood cells to bacterial products in vitro and measured the production of IL-6 and TNF-α in the context of varying concentrations of dexamethasone. This was done with a protocol originally developed by DeRijk (52) and modified for use by our group (37,53). Ten milliliters of blood were drawn into lithium-heparin tubes (Vacutainers; Becton-Dickinson), and within 1 hour diluted with saline 10:1. The diluted sample was then added to a 24-well flat-bottom plate in 800 μl aliquots. One hundred microliters of bacterial extract in the form of lipopolysaccharide (LPS; Sigma Chemical, Saint Louis, MO) were then added to each well at a final concentration of 100 ng/ml.[1] Next, 100 μl of the synthetic glucocorticoid dexamethasone (Sigma Chemical), dissolved in phosphate buffering solution, was added to each well. The dexamethasone was dissolved in varying quantities of phosphate buffering solution so that final in-well concentrations were 0, 1, 10, 50, 100, or 1000 nM. Samples were then incubated for 6 hours at 37°C with 5% CO_2. The plates were removed from the incubator and centrifuged for 10 minutes at 1000*g*. After the supernatants had been aspirated, they were frozen at −70°C until the study was completed. The inflammatory cytokines IL-6 and TNF-α were later measured using commercially available ELISAs (Becton-Dickinson Pharmingen, Heidelberg, Germany). The sensitivity of these kits is 3.2 pg/ml and 4.4 pg/ml, respectively, and the intra-assay coefficients of variation are 4.4 and 5.3%.

[1]This concentration of LPS is high. However, this is necessary in techniques like ours in which leukocytes are co-incubated with bacterial products and anti-inflammatory compounds. Without a pharmacologic concentration of LPS, the larger dosages of dexamethasone would suppress cytokine production below detectable levels.

These procedures yielded 2 values that were later used in statistical analyses. First, white blood cells' ability to produce inflammatory cytokines was represented by IL-6 and TNF-α values in LPS-treated cultures that did not contain dexamethasone. Because the whole blood assay we used does not account for disparities in the cellular composition of samples, cytokine values were corrected for the absolute number of monocytes in circulation at the time of the blood draw. (Monocytes produce the vast majority of inflammatory cytokines in LPS-stimulated cultures.) To facilitate interpretation and stabilize variance, corrected values were log-10 transformed before statistical analysis.

Second, white blood cells' sensitivity to the anti-inflammatory properties of glucocorticoids was estimated by generating a dose-response curve for each subject. We then calculated the concentration of dexamethasone needed to diminish cytokine production by 50%. This value is called the 50% inhibitory concentration (IC_{50}) and is widely used to model the potency of antagonist medications. IC_{50} calculations were performed in GraphPad Prism 3.02 (San Diego, CA). This software estimates the log IC_{50} (instead of the raw IC_{50}) because this value has superior statistical properties. Readers should note that log IC_{50} values are inversely proportional to glucocorticoid sensitivity. That is, higher IC_{50} values indicate that more dexamethasone is needed to suppress cytokine production by 50%, and thus white blood cells are viewed as more resistant to anti-inflammatory signals. All IC_{50} calculations were done on monocyte-corrected cytokine values. Log-10 transformations were used later to stabilize the variance of IC_{50} values.

Statistical Analyses

The primary objective of the statistical analyses was to determine whether depressed and control subjects exhibited different *response trajectories* to the stressor. Thus, we used an analysis of variance strategy to estimate a series of within-subjects contrasts reflecting biological responses over the course of the session. For each outcome, we report a within-subjects contrast for the effect of time, which reflects the extent of change for the whole sample over the lab session. We also report a contrast for the Group × Time interaction. This reflects the extent to which depressed and control patients exhibit different response trajectories over the session. Because our protocol was designed to assess both reactivity and recovery processes, many of the outcomes changed with the stressor and then returned to baseline values by the last blood draw. To capture the dynamics of this process, the contrasts we estimated and reported are quadratic trends. These values differ from the linear trends typically reported in research, in that they capture the shape of the response trajectory from baseline all the way through stressor and recovery. Finally, for each outcome we report a main effect of group, reflecting the extent of depression-control differences across the session. For all statistical analyses, α was set to 0.05, and 2-tailed tests of significance were used. Because of occasional technical difficulties with venipuncture and laboratory equipment, reported degrees of freedom vary slightly from analysis to analysis. All data are reported as mean ± SEM unless otherwise noted.

RESULTS

Preliminary Analyses

Table 1 displays the characteristics of the depressed and control subjects. With regard to demographic characteristics, the groups were similar in terms of age and ethnicity (p values $> .90$) but diverged on education and marital status, with depressed subjects having fewer years of schooling, $t(70) = 2.23$, $p < .03$, and a tendency to be single or divorced, $\chi^2 = 3.13$, $p < .08$. Scores on the Hamilton Rating Scale indicated that depressed subjects were, on average, in the midst of an episode of moderate severity (mean = 19.8; SEM = 0.94; range = 9–34). The majority had a history of major depressive episodes, and 25% were receiving treatment for their current mood problems, all in the form of psychotherapy. By design, none of the controls had current or previous depressive episodes. With regard to cardiovascular risk

TABLE 1. Characteristics of the Sample

	Depressed (N = 36)	Control (N = 36)
Demographic characteristics		
Age, years	26.6 ± 6.6	26.6 ± 6.6
Caucasians	18 (50.0%)	18 (50%)
African Americans	15 (41.7%)	15 (41.7%)
Asian Americans	3 (8.3%)	3 (8.3%)
Education, years	14.3 ± 2.0	15.1 ± 1.9
Married	0 (0.0%)	3 (8.3%)
Divorced/separated	5 (13.9%)	2 (5.6%)
Depression characteristics		
Hamilton score, 17-item	19.8 ± 5.6	0.5 ± 1.2
Current episode length, weeks	33.1 ± 46.8	—
History of previous episodes	24 (66.0%)	0 (0.0%)
Number of previous episodes	3.1 ± 4.6	—
Currently in psychotherapy	9 (25.0%)	—
CHD risk factors		
Daily smokers	12 (33.3%)	3 (8.3%)
Alcoholic drinks, per week	2.8 ± 5.1	3.5 ± 5.9
Body mass index, kg/m^2	27.9 ± 11.0	27.3 ± 7.5
Oral contraceptive user	6 (16.7%)	17 (47.2%)
Systolic Blood Pressure, mm Hg	113.7 ± 12.8	112.9 ± 9.1
Diastolic blood pressure, mm Hg	66.7 ± 11.1	66.8 ± 6.3
Resting heart rate, beats per minute	68.7 ± 9.4	66.5 ± 6.8
Family history of premature CVD	9 (25.0%)	3 (8.3%)

Values are mean ± SD or number of subjects (% of sample).
CHD = coronary heart disease; — = not applicable; CVD = cardiovascular disease.

factors, the groups were similar in terms of alcohol use, body mass index, systolic and diastolic blood pressure, and resting heart rate, t values > 1.15, p values $> .26$. However, depressed subjects were more likely than controls to be daily smokers, $\chi^2 = 6.82$, $p < .01$, and less likely to be using oral contraceptive medications, $\chi^2 = 7.73$, $p < .01$. They were also marginally more likely to have a family history of premature cardiovascular disease, $\chi^2 = 3.60$, $p < .06$.

Psychological Responses

Analyses revealed that the mock job interview successfully elicited negative emotions. Subjects reported significant increases in anxiety and shame from before to after the stressor protocol, F values $(1,70) > 68.38$, p values $< .001$. These changes were quite large on average, with increases of 80 to 90% across the sample. The Group × Time interactions were nonsignificant, however, indicating that depressed and control subjects showed equivalent increases in these emotions, F values < 1, p values $> .70$. The sample as a whole did not report reliable changes in anger or sadness as a function of the job interview, F values < 1.3, p values $> .30$, and there were no significant Group × Time interactions, F values < 1, p values $> .74$ for these emotions. Together, these findings indicate that the interview markedly increased anxiety and shame in subjects but did not affect their level of anger or sadness.

Cortisol Responses

Analyses revealed that salivary cortisol declined in linear fashion over the course of the session, $F(1,68) = 19.58$, $p < .001$ (Table 2). This pattern was not surprising to us, as lab sessions occurred between 8:00 AM and 10:00 AM, when cortisol levels are rapidly declining from their early morning peak. Despite this pattern, there was a significant Group × Time interaction ($F(1,68) = 3.94$, $p = .05$), indicating the groups had different response trajectories. Among control subjects, cortisol increased slightly following the stressor and then declined over the rest of the session. By contrast, cortisol declined steeply over the session in depressed subjects, without any stressor-related boost. There was no overall cortisol difference between depressed and control subjects across the session, $F(1,68) = 1.93$, $p > .17$ and no evidence of a difference under resting conditions at baseline, $t < 1$, $p > .47$. Together, these findings suggest the possibility of a blunted cortisol response to stress among depressed subjects. However, this interpretation is complicated somewhat by the timing of our lab sessions, which allowed the normative diurnal decline to obscure stress-related cortisol increases.

Immune Responses

Leukocyte Subsets

There was a marked change in leukocyte subset numbers as a function of the stressor (Table 3). Counts of leukocytes, neutrophils, and monocytes increased from before to after the stressor and generally declined toward baseline during the 30-minute recovery period. These effects were evident in the significant quadratic contrasts for Time: for leukocytes, $F(1,60) = 37.01$, $p < .001$; for neutrophils, $F(1,60) = 8.83$, $p < .004$; for monocytes, $F(1,60) = 38.72$, $p < .001$. Lymphocyte numbers did not change reliably over the session, $F(1,60) = 1.61$, $p > .21$. There was no evidence of differential leukocyte responses to the stressor among depressed and control subjects, for Group × Time interactions, F values < 1, p values $> .38$. There also was no overall difference in

TABLE 2. Salivary Cortisol Over the Course of the Session

	Baseline (−30 min)	PreTask (−1 min)	Post-Task (+17 min)	Recovery (+27 min)	Recovery (+37 min)	Recovery (+47 min)
Depressed subjects (N = 36)	12.39 (11.19)	10.68 (10.51)	9.84 (10.86)	7.82 (7.65)	7.26 (5.96)	7.30 (5.66)
Control subjects (N = 36)	13.07 (10.68)	12.78 (11.27)	14.29 (9.52)	11.99 (8.50)	10.42 (6.45)	9.73 (6.49)

Values are group means with standard deviations in parentheses. Units are nmol/L.

TABLE 3. Immune Parameters Before, During, and After Stressor Exposure

	Depressed Subjects ($N = 36$)			Control Subjects ($N = 36$)		
	Baseline	Post-Stress	Recovery	Baseline	Post-Stress	Recovery
Leukocytes ($\times 10^9$ cells/L)	6.17 (1.26)	6.53 (1.44)	6.41 (1.40)	6.22 (1.68)	6.69 (1.74)	6.31 (1.86)
Neutrophils ($\times 10^9$ cells/L)	3.68 (1.08)	3.76 (1.32)	3.77 (1.38)	3.70 (1.56)	3.93 (1.62)	3.81 (1.74)
Monocytes ($\times 10^9$ cells/L)	0.37 (0.12)	0.43 (0.13)	0.38 (0.12)	0.39 (0.14)	0.46 (0.12)	0.40 (0.12)
Lymphocytes ($\times 10^9$ cells/L)	1.89 (0.52)	2.15 (0.96)	2.23 (1.19)	2.00 (0.53)	2.14 (0.52)	1.96 (0.50)
C-reactive protein (mg/L)	3.33 (4.12)	3.49 (4.32)	3.57 (4.36)	3.30 (4.13)	3.59 (4.44)	3.49 (4.38)
Circulating IL-6 (pg/ml)	1.79 (2.33)	1.80 (2.52)	1.86 (2.16)	2.06 (2.46)	2.12 (3.02)	2.06 (2.46)
Circulating TNF-α (pg/ml)	5.98 (6.82)	4.96 (4.62)	7.23 (9.6)	6.16 (5.32)	6.49 (5.94)	5.74 (5.76)
IL-6 production (log-10; pg/ml)	4.88 (0.18)	4.90 (0.18)	4.91 (0.24)	4.88 (0.18)	4.90 (0.16)	4.91 (0.18)
TNF-α production (log-10; pg/ml)	3.81 (0.24)	3.83 (0.25)	3.92 (0.23)	3.81 (0.24)	3.84 (0.36)	3.91 (0.17)
IC_{50} IL-6 (log-10)	1.29 (0.05)	1.31 (0.04)	1.29 (0.06)	1.31 (0.05)	1.30 (0.04)	1.31 (0.05)
IC_{50} TNF-α (log-10)	1.28 (0.06)	1.31 (0.05)	1.28 (0.06)	1.31 (0.05)	1.29 (0.05)	1.31 (0.06)

Values are group means with standard deviations in parentheses. Note that values for IL-6 production, TNF-α production, and IC_{50} have been corrected for numbers of circulating monocytes.

leukocyte subset numbers between depressed and control subjects across the session, F values < 1, p values $> .65$. Collectively, these findings suggest that the stressor was associated with a transient mobilization of leukocytes, particularly neutrophils and monocytes, that was similar in magnitude between depressed and control subjects.

Circulating Inflammatory Molecules

Analyses revealed that C-reactive protein levels increased from before to after the stressor and then declined toward baseline during the recovery period (Table 3). This effect was evident in the significant quadratic contrast for Time, $F(1,60) = 10.88$, $p < .002$. There also was a marginally significant Group $\times$ Time interaction, $F(1,60) = 3.12$, $p < .08$, indicating that the groups had different response trajectories. Among control subjects, C-reactive protein increased following the stressor and then declined partway to baseline during recovery. By contrast, depressed subjects exhibited a smaller increase following the stressor, but their C-reactive protein levels continued to rise during recovery. Their peak level at the end of recovery was nearly identical to the controls' value after the stressor. Overall, subjects did not show reliable changes in IL-6 and TNF-α concentrations over the session, F values < 1, p values $> .44$, and there was no evidence of a differential response trajectory for these molecules between the groups, F values < 1.6, p values $> .21$. These findings suggest that acute stress boosts C-reactive protein levels in blood. Although this process gets partially reversed within 30 minutes among control subjects, it continues through the recovery period in those who are clinically depressed.

Regulation of Inflammatory Cytokines

There was a significant increase in stimulated cytokine production as a function of the stressor (Table 3). Supernatant concentrations of IL-6 and TNF-α rose from before to after the stressor and then continued increasing during the recovery period, $F(1,64) = 9.56$, $p < .003$ and $F(1,64) = 29.92$, $p < .001$, respectively. There was no evidence that cytokine production changed differentially among depressed and control subjects, for Time $\times$ Group interactions, F values < 1.0, p values $> .40$. There also was no overall difference in IL-6 or TNF-α production between the groups, F values < 1.0, p values $> .39$. Thus, although the stressor was followed by increased production of inflammatory cytokines, the magnitude of this effect was similar in depressed and control subjects.[2]

Exposure to the stressor also influenced white blood cells' sensitivity to the anti-inflammatory properties of glucocorticoids. Although the contrasts for Time were not significant for either IL-6 or TNF-α IC_{50} values, F values < 1.0, p values $> .43$, inspection of the Group $\times$ Time interactions revealed immediately why this was the case. With both molecules, depressed and control subjects exhibited different response trajectories over the session, for IL-6, $F(1,61) = 8.88$, $p < .001$; for TNF-α, $F(1,67) = 4.47$, $p < .04$. Depressed subjects began the session with higher glucocorticoid sensitivity than controls subjects. Following exposure to the stressor protocol, however, sensitivity declined among depressed subjects and increased among control subjects. IC_{50} values for both cytokines generally returned to baseline by the end of the recovery period. These findings are depicted in Figure 1. There were no overall differences in IL-6 or TNF-α glucocorticoid sensitivity between the groups, F values < 2.0, p values $> .17$.[3]

[2] An identical pattern of findings emerged when analyses were conducted using cytokine volumes that were not adjusted for monocyte count. For both IL-6 and TNF-α, supernatant concentrations rose from before to after the stressor and continued increasing during recovery, $F(1,64) = 21.58$, $p < .001$ and $F(1,64) = 27.97$, $p < .001$. There was no evidence that cytokine production changed differentially among depressed and control subjects (for Time $\times$ Group interactions, F values < 1.0, p values $> .40$) and no overall difference in the production of either cytokine between the groups (F values < 1.0, p values $> .39$).

[3] Again, an identical pattern of findings emerged when analyses were conducted using IC_{50} values that were not adjusted for monocyte count. Depressed and control subjects had different response trajectories for both IL-6 and TNF-α: $F(1,61) = 4.77$, $p < .03$ and $F(1,63) = 4.37$, $p < .04$. There was no main effect of time (F values < 1.0, p values $> .43$) and no main effect of diagnosis (F values < 2.06, p values $> .16$) for either outcome.

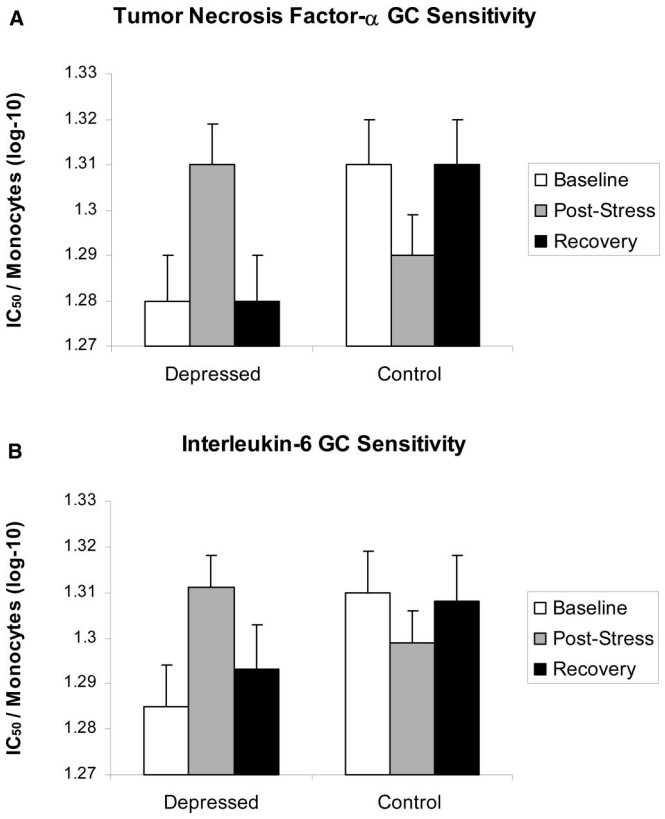

Figure 1. Mean IC_{50} values for IL-6 and TNF-α during stressor as a function of diagnosis (error bars represent standard error of mean). This figure shows that depression moderates the impact of acute stress on the immune system's sensitivity to the anti-inflammatory properties of glucocorticoids. This measure reflects the capacity of dexamethasone to inhibit the in vitro production of IL-6 and TNF-α. Depressed subjects began the session with higher sensitivity to dexamethasone's anti-inflammatory properties than control subjects. Following exposure to the stressor protocol, however, sensitivity declined among depressed subjects and increased among control subjects. Sensitivity returned to baseline by the end of the recovery period for both cytokines.

Ruling Out Confounds

Earlier we noted that depressed and control subjects differed on a number of demographic and medical characteristics. To determine whether these factors contributed to the observed disparities in biological response to the stressor, we reconducted statistical analyses in which depressed and control subjects showed divergent trajectories, each time statistically adjusting (covarying) for education, marital status, smoking, oral contraceptive use, or family history of premature heart disease. The 3 outcomes on which the groups showed different response trajectories were salivary cortisol, IL-6 IC_{50} value, and TNF-α IC_{50} value. The addition of covariates did not alter the Group $\times$ Time interactions for cortisol or IL-6; all continued to be statistically significant with p values remaining $< .05$. For the TNF-α IC_{50} value, the same was generally true, because interactions remained statistically significant with p values $< .04$. There were 2 exceptions to this rule: when smoking status and family history were included as covariates, they each reduced the interaction to marginal significance at $p < .08$. (Not surprisingly, covarying out other demographic characteristics or coronary heart disease risk factors did not alter the findings, depressed and control subjects did not differ on these factors to begin with.) Overall, these findings suggest that demographic and medical differences explain little (if any) of the disparity in stressor response between depressed and control subjects.

DISCUSSION

The mock interview produced a variety of behavioral and biological alterations across our sample of healthy young adults. Subjects' levels of anxiety and shame increased markedly, rising by 80 to 90% from their typical intensity. This pattern of emotional response is similar to what has been observed in other public speaking paradigms, where subjects primarily experience fears of negative social evaluation and corresponding increases in self-conscious emotion. It is these feelings of shame and anxiety, rather than anger and sadness, that seem to activate the hypothalamic-pituitary-adrenocortical axis and cytokine network during acute stress (54,55). The stressor also evoked a transient increase in circulating numbers of leukocytes, neutrophils, and monocytes that partially reversed by the end of the recovery period. The effect sizes associated with these increases ranged from $r = 0.10$ to $r = 0.22$, figures that closely resemble what has been found in other studies of the immune response to acute stress (36). Levels of C-reactive protein rose slightly during the stressor (by 7.5%, effect size of $r = 0.09$); however, circulating concentrations of IL-6 and TNF-α did not change reliably. These findings are consistent with the emerging literature on circulating inflammatory markers, which generally documents little or no change in these molecules during or shortly after acute stressors (36). It is possible that with a longer recovery period, reliable increases in cytokine concentrations might have been observed, as has been the case in some research (56,57). With regard to immune regulation, the stressor was associated with increases in the stimulated production of IL-6 and TNF-α, a process that continued through the end of the recovery period. The effect sizes associated with these increases ranged from $r = 0.15$ to $r = 0.23$, which again is similar in direction and magnitude to previous research on acute stressors (36).

There were several domains in which the stressor evoked disparate patterns of response among depressed and control subjects. This was partially evident in the analyses of salivary cortisol. Whereas control subjects exhibited a small boost in this hormone at the outset of the job interview, depressed subjects showed a steady decline over the course of the lab session. This pattern of findings suggests that depression may be characterized by a blunted cortisol response to acute challenge. That said, we recognize that our conclusions must be tempered somewhat, as even control subjects did not show a marked cortisol boost during the stressor. However, we are inclined to believe that this boost was simply obscured by the

strong diurnal rhythm of cortisol in the morning hours,[4] and would have emerged had the lab sessions been held in the afternoon and evening hours, when such effects are typically detected (54).

Our confidence that depression is accompanied by a blunted cortisol response to challenge is bolstered by 2 additional considerations. First, other studies have documented this pattern, in both lab and field settings (41,42). Moreover, a recent meta-analysis reported that across the published literature, there was a significant reduction in cortisol response to stress among depressed people (43). Second, we also assessed the cortisol response to awakening in this sample of women and found that it was reduced by a full standard deviation in depressed subjects (58). Collectively, these findings suggest that depression may be accompanied by a blunted cortisol response to both mental and physical challenge, and one that is apparent across clinic and field settings. The clinical significance of this blunting is uncertain. However, cortisol plays a critical role in regulating the magnitude and duration of the immune response, so it will be important for future research to better characterize this pattern and evaluate its clinical relevance.

We also detected a different response trajectory for white blood cells' sensitivity to the anti-inflammatory properties of glucocorticoids. Following exposure to the mock job interview, sensitivity declined among depressed subjects, whereas it increased among control subjects. This pattern emerged for both IL-6 and TNF-α, suggesting that it is a general phenomenon that cuts across inflammatory outcomes. What implications might this finding have for the development or progression of disease? If depressed individuals' glucocorticoid sensitivity was diminished over the long-term through exposure to repeated stressors, this would likely facilitate the sustained expression of inflammatory mediators. A process of this nature could foster a number of adverse disease outcomes. Among patients with cardiac disease, for example, it could lead to acute complications such as plaque rupture, thrombus formation, and sudden cardiac death (15,59). And among patients who suffer from autoimmune conditions, it could promote tissue damage and symptom flare-ups (14,17). That said, further research is needed to evaluate whether this chain of events might underlie any of the excess morbidity and mortality in depressed populations.

It bears noting that during the baseline period, depressed subjects exhibited higher glucocorticoid sensitivity than controls. Though this finding might seem inconsistent with previous research, which shows that depression is generally associated with resistance to glucocorticoids (35,60,61), there are several reasons why it may make sense in this context. First, previous research has focused heavily on patients with severe depression. These patients are likely to exhibit elevations in cortisol, which over time leads to a decline in receptor sensitivity for glucocorticoids (62,63). By contrast, the patients in our sample were suffering from depression of mild-moderate severity and were characterized by blunted cortisol responses to stress and waking (58). Under these conditions, white blood cells would be expected to show enhanced sensitivity to glucocorticoids, because they are exposed to low concentrations of these molecules in vivo. Compensatory processes of this nature have been seen in other groups with blunted cortisol output, such as patients with post-traumatic stress disorder and women who use oral contraceptive medications (38,39). Second, we have observed a similar pattern in another sample of patients with mild-moderate symptoms of depression (34). In this cohort of patients recovering from an acute coronary syndrome, depressive symptoms were also associated with heightened sensitivity to glucocorticoids at rest.

Although this is a plausible explanation for the baseline findings, it does not provide insight into what occurred during the stressor. Specifically, it is unclear why depressed people would exhibit reductions in cortisol secretion *and* glucocorticoid sensitivity after exposure to acute stress. If cortisol is the mechanism through which stress modulates glucocorticoid sensitivity, one would expect these effects to run in opposite directions (i.e., for greater boosts in cortisol secretion to occur in parallel with declines in glucocorticoid sensitivity). But in the current sample, this pattern did not emerge. Perhaps some other biological product, such as catecholamines, played a role in shaping these processes. Regardless, further research is needed to understand the complex regulation of the inflammatory response and how it is modulated by factors related to context (resting versus stress) and person (healthy versus depressed).

We also found marginal evidence of a differential response trajectory for C-reactive protein. Control subjects exhibited an increase in this molecule following the stressor, which partially reversed itself by the end of the recovery period. By contrast, depressed subjects exhibited a smaller boost following the stressor, but their C-reactive protein continued to rise during recovery so that by the end it had peaked near the same level as controls. Although these different trajectories may prove to be interesting in the future, we are reluctant to offer any definitive conclusions about them at present. There are several reasons for our caution. First, the Group $\times$ Time interaction was only marginally significant, and it will need to be replicated before we can be fully confident that it is robust. Second, even if this effect proves to be reliable, it seems to be a difference in timing rather than magnitude. The peak level of C-reactive protein was almost identical in both groups; it simply took more time for depressed subjects to get there. Without a reliable disparity in magnitude, it becomes difficult to use these findings as an explanation for the greater morbidity and mortality observed in depressed people. Finally, any boost in C-reactive protein that occurs over the course of

[4]Though we believe that the timing of lab sessions is the major issue here, the small cortisol boost among controls could stem from other factors as well. For instance, we included women at all phases of the menstrual cycle as well as those taking oral contraceptives. Both of these factors are associated with a smaller cortisol response to acute stress (39,67). We also use a modified version of the Trier Social Stress Test toxin, and it is possible that the alterations reduced its ability to evoke a large cortisol response. This explanation strikes us as somewhat implausible, however, given the wide variety of behavioral and biological alterations that occurred after the task.

an acute stress paradigm is unlikely to reflect de novo production of this molecule. To actually increase the volume of C-reactive protein in the body, an acute stressor would first need to evoke production of IL-6, which would then signal the liver to synthesize and release C-reactive protein (64). This process would take at least 1 to 2 hours to unfold, because it would involve transcription and translation of multiple molecules. In most research that has been done on acute stress, the lab session would be long over before any new C-reactive protein appeared in circulation. The stress-evoked increase that was observed in our sample (and others, as well) more likely reflects C-reactive spillover from tissue stores, the liver, or immune organs. In this way, it is much like the transient redistribution of white blood cells that occurs during acute stress, from marginating pools and lymphoid organs into the peripheral circulation (40,65,66).

The study had several limitations that merit discussion. First, it did not include a control condition that would allow us to rule out alternative explanations for stress-related change, such as the passage of time or repeated blood draws. This limitation is particularly salient for outcomes that have robust diurnal variations such as cortisol; any change in these processes could be related to advancing circadian rhythms rather than acute stress. We view this as less of a problem for outcomes like glucocorticoid sensitivity, where it is difficult to imagine how extraneous factors like time passage could give rise to opposite response trajectories among depressed and control subjects. Second, our technique for assessing glucocorticoid sensitivity used dexamethasone, a synthetic and especially potent version of the endogenous hormone cortisol. We used this technique because it has been validated previously and is known to be sensitive to the effects of stressors and depression (34,37–39,63). However, questions remain about the in vivo significance of assays that use dexamethasone, and research that employs cortisol as an anti-inflammatory compound is needed. Finally, our study enrolled physically healthy young adults but not patients suffering from cardiac, autoimmune, or other diseases. Thus, it is not clear whether our findings would extend to medical patients with depression and, even if they do, whether stress-related increases in glucocorticoid sensitivity would have the clinical implications about which we speculate. It will be important for future research to address these questions in patients with medical conditions that involve excessive inflammation. Nevertheless, these findings provide initial insights into how depression modifies the inflammatory response to acutely challenging situations, an issue that has not yet been addressed in the literature. With further progress in this area, researchers may gain valuable insights into the processes through which depression "gets inside the body" to influence such a broad array of medical conditions.

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