Pathways Linking Major Depression and Immunity in Ambulatory Female Patients

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Objectives: The goals of this study were to investigate whether depression is associated with cellular immunity in ambulatory patients and to identify neuroendocrine and behavioral pathways that might account for this relationship. **Methods:** We studied 32 women who met *Diagnostic and Statistical Manual of Mental Disorder*, fourth edition, criteria for major depressive disorder and 32 healthy female control subjects. The groups were matched for age and ethnicity. None were taking medication, and all were free of disease involving the immune system. **Results:** Depressed subjects had reduced proliferative responses to the mitogens concanavalin A and phytohemagglutinin compared with control subjects. Natural killer cell activity was reduced among older depressed subjects but enhanced among younger depressed subjects. Although depression was associated with elevated circulating levels of norepinephrine and estradiol, these hormones could not account for the immunologic differences between depressed and control subjects. Depression was also associated with greater tobacco and caffeine consumption, less physical activity, and poorer sleep quality. Mediational analyses were consistent with physical activity acting as a pathway through which depression was associated with reduced lymphocyte proliferation. **Conclusions:** Ambulatory patients with mild to moderately severe depression exhibit reduced mitogen-stimulated lymphocyte proliferative responses and altered natural killer cell cytotoxicity. The relationship between depression and proliferative responses may be mediated by physical activity. **Key words:** psychoneuroimmunology, depression, immunity, physical activity.

ACTH = adrenocorticotropin; ANOVA = analysis of variance; BDI = Beck Depression Inventory; ConA = concanavalin A; CV = coefficient of variation; DSM-IV = *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition; ELISA = enzyme-linked immunosorbent assay; GCRC = General Clinical Research Center; HAM-D = Hamilton Rating Scale for Depression; HPA = hypothalamic-pituitary-adrenal; IL-1β = interleukin 1-β; NKCC = natural killer cell cytotoxicity; PHA = phytohemagglutinin; POMS = Profile of Mood States; SADS-L = Schedule for Affective Disorders–Lifetime Version; SCID-P = Structured Clinical Interview for DSM-IV–Patient Version; THC-tetrahydrocannabinol.

INTRODUCTION

The immune systems of clinically depressed patients are different from those of healthy control subjects (1). Depressed individuals show reduced NKCC, blunted mitogen-stimulated lymphocyte proliferative responses, and suppressed delayed-type hypersensitivity reactions (2–5). There are, however, a number of questions that remain about immune differences between depressed and normal individuals. These include the possibility that hospitalization contributes to this effect, that differences occur only among severely depressed patients, and that the associations differ across patients of different ages. Most importantly, there is still little understanding of why such an association exists.

The vast majority of research on depression and immunity has focused on hospitalized patients. This is problematic for several reasons. First, only a small minority of individuals suffer from depressive episodes that are severe enough to warrant inpatient treatment (6). It is thus unclear to what extent depression-related immune alterations would be generalizable to most clinically depressed individuals. Second, hospitalization itself could induce immunologic changes by altering patterns of sleep, diet, and physical activity (7). Thus, it is difficult to ascertain to what extent immune alterations are a consequence of the depression per se or are attributable to the experience of hospitalization.

Several investigations have attempted to circumvent these problems by studying ambulatory patients with mild to moderately severe cases of depression. These studies have yielded inconsistent findings. Some have demonstrated that depression is associated with reduced cellular immune response (8, 9), others have demonstrated that it is associated with increased response (10–12), and still others have found no differences between depressed patients and control subjects (13, 14). Some of this disparity in outcomes may stem from the inclusion of control subjects with a positive psychiatric history or depressed patients with comorbid psychiatric disorders. It also could arise from differences across studies in statistical power or the extent of error involved in measuring immune parameters using in vitro assays with peripheral blood lymphocytes.

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Preliminary evidence indicates different associations between depression and immunity in younger vs. older adults. Among older adults, depression is associated with blunted lymphocyte proliferative responses and fewer circulating CD4$^+$ T lymphocytes. In contrast, younger adults who are depressed show a trend toward more pronounced proliferative responses and higher CD4$^+$ counts (4, 13). One possible explanation is that these differences occur because younger and older depressed patients differ in endocrine regulation and response. For instance, with advancing age, depression is more likely to be accompanied by basal cortisol hypersecretion and cortisol nonsuppression after dexamethasone administration (15–17). It is also possible, however, that the health practices of younger and older patients differ or that poor health practices among older patients are more detrimental than they are among the young.

Finally, there is little evidence at this point indicating why depressed patients have altered immune systems (1). One hypothesis proposes that neuroendocrine mechanisms are responsible. Clinical depression can be accompanied by dysregulation of the HPA axis, the sympathetic-adrenal-medullary axis, and the hypothalamic-pituitary-ovarian axis (18, 19). Receptors for the hormonal products of these systems have been identified on lymphocytes, and ligation of those receptors induces changes in lymphocyte function (20–22). An alternative hypothesis is that health practices are responsible for immunologic differences. Depressed and nondepressed may have different patterns of tobacco use, caffeine use, physical activity, nutrition, and sleep (23, 24). All of these health practices can alter immune response (25–28).

Depressed patients with dysregulation of the HPA axis have increased production of proinflammatory cytokines and suppressed lymphocyte proliferative responses (29, 30). However, it is not clear on the basis of these studies whether HPA function can account for the immunologic differences *between* depressed and nondepressed individuals. Studies that have taken this approach have found that levels of hormones such as cortisol, ACTH, epinephrine, and norepinephrine do not explain immunologic differences between depressed and nondepressed individuals (4, 8, 12, 31). Research examining the mediational role of health practices has also yielded negative findings. Specifically, the implementation of statistical controls for smoking, alcohol and caffeine consumption, weight change, and nutrition does not attenuate the association between depression and immune parameters (3, 4, 8, 11–13). Irwin et al. (25, 32) showed that sleep disturbances can modulate NKCC in both depressed and nondepressed individuals but did not evaluate whether sleep disturbances could account for differences between depressed patients and control subjects. To date, no research has considered whether physical activity might act as a mediator of the link between depression and immunity, despite evidence from the animal literature that exercise can provide a buffer against stress-induced downregulation of NKCC (33).

In the present study, we assessed immune parameters in two diagnostically homogenous groups: 1) ambulatory patients with clinical depression but no other psychiatric disorder and 2) healthy control subjects without a history of psychiatric illness. We also assessed immune parameters on two occasions to minimize the random error associated with measurement and hence increase statistical power. To further elucidate age-related differences in the relationship between depression and immunity, we studied depressed women both below and above 40 years of age, the age at which the association between depression and immunity is thought to switch direction (4). Finally, we examined a broad spectrum of potential pathways through which depression might be associated with immunity. These included endocrine (epinephrine, norepinephrine, cortisol, estradiol, and progesterone) and behavioral (tobacco use, alcohol and caffeine consumption, physical activity, sleep hygiene, and nutrition) pathways. We also examined whether different pathways might be operative for younger and older patients.

METHODS

Participants

The participants were 32 women with major depressive disorder and 32 control women without a lifetime history of psychiatric disorder. All subjects were judged to be in good health after a physical examination, with no medical condition involving the immune system and no current use of medication with the exception of oral contraceptives. Women were excluded from participation if they were 1) pregnant or breast-feeding; 2) menopausal, postmenopausal, or had irregular menstruation; 3) undernourished as evidenced by abnormal levels of serum albumin, transferrin, or retinol-binding protein; 4) displayed evidence of anemia on complete blood count; or 5) had toxicology screens positive for amphetamines, barbiturates, benzodiazepines, methadone, opiates, phencyclidine, or cocaine or THC metabolites. Depressed and control subjects were matched for age, ethnicity, and oral contraceptive use.

The depressed subjects were recruited from an ongoing study of the prophylactic value of maintenance psychotherapy. Assessments for the present study were completed before subjects began treatment. All subjects met DSM-IV criteria for major depressive disorder without psychotic features. Diagnoses were made by psychiatric research nurses using the SCID-P (34) or the SADS-L (35) in conjunction with research diagnostic criteria. Depression severity was assessed using the 17-item version of the HAM-D (36). Those with

comorbid axis I disorders or a history of psychiatric illness other than depression were excluded. Physical examinations with appropriate laboratory tests were performed to exclude cases of depression that were secondary to organic causes or medical illness. Subjects had not used antidepressant medication for at least 6 months before study entry. One subject reported intermittent use of diazepam and alprazolam until 1 month before she began the study. All subjects were being treated on an outpatient basis.

Control subjects were recruited through advertisements in local media. On the basis of psychiatric screening interviews (SCID-P or SADS-L) with research nurses, none met DSM-IV criteria for either a current or lifetime axis I psychiatric disorder. None reported a history of using antidepressant or neuroleptic medications. Two subjects reported histories of intermittent diazepam use; however, both had discontinued the medication more than 10 years before study entry. One control subject had abnormally low serum levels of the nutritional markers albumin, transferrin, and retinol-binding protein. She and her matched counterpart in the depressed group were therefore excluded from the analyses reported here. Including these subjects in analyses yielded identical results.

Procedures

Subjects who met eligibility criteria for the study were scheduled for a laboratory session at the GCRC of the University of Pittsburgh School of Medicine, Pittsburgh, PA. Sessions took place between 7:00 AM and 10:00 AM to control for diurnal variations in endocrine and immune parameters. Although logistical difficulties precluded us from studying depressed subjects and their matched counterparts in the control group on the same day, pairs' sessions always took place at the same hour of the day. Subjects abstained from food, drink, tobacco, and strenuous exercise for 1 hour before sessions. On arrival at the GCRC, subjects completed paper-and-pencil questionnaires, including the BDI (37) and the POMS (38). An indwelling catheter was then inserted into the antecubital fossa of one arm, and subjects rested quietly for 45 minutes. At the end of the acclimation period, 120 ml of blood was drawn into ethylenediaminetetraacetic acid- or heparin-coated Vacutainer tubes. The entire laboratory session lasted approximately 90 minutes. Subjects returned to the GCRC 1 week later at the same time of day for a second session. Identical procedures were followed, and identical data were collected. During one of the weekdays between GCRC sessions, subjects provided a 24-hour urine sample for the determination of urinary catecholamines and cortisol. Because of scheduling and technical difficulties, eight subjects (four depressed and four control) had data from only one of the two GCRC sessions. For these subjects, neuroendocrine and immune parameters were calculated on the basis of a single blood draw.

After GCRC sessions, samples were divided into separate aliquots for the various biological assays. Specimens were then delivered to the appropriate laboratory for analysis. Immunologic assays were conducted on the same day that venipuncture occurred. Neuroendocrine samples were frozen at −70°C, and assays were performed in a single batch. Technicians who conducted neuroendocrine and immunologic assays were blind to subjects' depression status. This protocol was approved by the institutional review boards of the University of Pittsburgh School of Medicine and Carnegie Mellon University, Pittsburgh, PA. All subjects provided written informed consent and received $40 for their participation in each session.

Assessment of Pathways Linking Depression And Immunity

Health practices. Health practices were measured with an inventory that has been used in other published research from this laboratory. It has demonstrated excellent psychometric properties in those studies (39, 40). Participants were classified as smokers if they smoked cigarettes, cigars, or pipes on a daily basis (40). Alcohol use was determined by counting the number of alcoholic drinks consumed during the previous week. A drink was considered a bottle or can of beer, a glass of wine, or a shot of hard liquor (40). Caffeine intake was measured as the number of caffeinated beverages consumed during the previous week. Subjective sleep quality (rated on a scale of 1–4), sleep duration, and sleep efficiency were assessed with the Pittsburgh Sleep Quality Index (41). Sleep efficiency is the proportion of time in bed a participant spends sleeping. Physical activity was assessed by asking participants how often they engaged in aerobic exercise, walking or jogging, and physical work around the house. Response options were never or infrequently (0), sometimes (1), or frequently (2). A physical activity index was formed by summing responses to the three items.

The test-retest reliability of the health practice inventory was assessed by having a subsample of 17 control subjects complete it a second time 6 months after the initial administration. Because of the long lag between tests, this provides a very conservative assessment of measurement reliability. Smoking status did not change in any of the subjects. Correlations between the two administrations were 0.93 for alcohol consumption, 0.75 for caffeine consumption, 0.76 for sleep duration, 0.78 for sleep efficiency, and 0.65 for the physical activity index (for all, $p < .005$). The correlation between subjective sleep quality assessments was only 0.23. This was not unexpected given the instability of subjective evaluations across extended periods of time. Even so, 11 of the 17 subjects reported identical responses at both times.

Body mass and nutrition. Body mass index was computed as weight in kilograms divided by height in meters squared. Nutritional status was assessed using serum levels of albumin, transferrin, and retinol-binding protein. These are markers of protein store depletion, iron deficiency, and retinol depletion, respectively. Albumin was measured using spectrophotometry. Transferrin and retinol-binding protein were assessed using rate nephelometry. Blood for these assays was drawn during the initial GCRC session.

Neuroendocrine parameters. Circulating and urinary levels of epinephrine and norepinephrine were determined using high-performance liquid chromatography with electrochemical detection. The interassay CVs were 1.49% and 3.49% for urinary norepinephrine and epinephrine, respectively, and 7.11% and 6.75% for plasma values. Cortisol was measured using double-antibody competitive radioimmunoassays. CVs were 10%, 12%, and 15%, for the plasma, urinary total, and urinary free cortisol assays, respectively. Progesterone and estradiol were assessed using chemoluminescence on a Chiron ACS180 instrument. CVs for both assays were 7%. To obtain a maximally reliable assessment of basal circulating neuroendocrine parameters, values obtained at the two GCRC sessions were averaged. Log 10 transformations were performed on norepinephrine values to normalize positively skewed distributions. The correlation between the two measurements was 0.76 for epinephrine, 0.41 for norepinephrine, and 0.64 for cortisol. Reproductive and urinary hormones were measured on only one occasion.

Measurement of Immune Parameters

Circulating leukocyte subset counts. Circulating numbers of leukocytes, granulocytes, monocytes, and lymphocytes were deter-

mined with white blood cell and differential counts using standard techniques. Lymphocyte subset, natural killer cell, and B cell counts were determined using flow cytometry with two-color immunofluorescence. Monoclonal antibodies labeled with phycoerythrin or fluorescein isothiocyanate were used to enumerate total T lymphocytes ($CD3^+$), helper T lymphocytes ($CD3^+/CD4^+$), cytotoxic/suppressor T lymphocytes ($CD3^+/CD8^+$), B cells ($CD3^-/CD19^+$), and natural killer cells ($CD3^-/CD16^+56^+$). Nonspecific binding was assessed using isotype controls labeled with phycoerythrin or fluorescein isothiocyanate. Cell counts were averaged across the two blood draws. Correlations between the two assessments were high (average $r = 0.73$; range = 0.53–0.83).

Mitogen-stimulated lymphocyte proliferation. Lymphocyte proliferation was assessed using whole blood diluted 1:10 in supplemented RPMI-1640. One hundred microliters of the diluted blood was added to 96-well, flat-bottom culture plates containing either 100 μl of control media, ConA (at doses of 1.00, 2.50, 10.00, or 20.00 μg/ml), or PHA (at doses of 0.50, 2.50, 10.00, or 20.00 μg/ml). Each assay was performed in triplicate. Plates were incubated for 4 days at 37°C in air and 5% CO_2. After incubation, wells were pulsed with 1 μCi of ^{3}H-thymidine. Cultures were incubated for an additional 18 hours, at which time cells were harvested for counting. Proliferative responses are expressed as the difference in counts per minute between stimulated and unstimulated wells (Δcpm).

For analyses examining whether proliferative responses differ across depressed and control subjects, we present data for the entire titration series for both mitogens. For the sake of economy, mediational analyses are based on peak responses to the mitogens (at 10 μg/ml for ConA and 2.50 μg/ml for PHA; see Ref. 42). Similar conclusions emerged when mediational analyses were conducted at other mitogen concentrations. Proliferative responses at the two GCRC sessions were strongly correlated for ConA (average r across four doses = 0.73; range = 0.61–0.80) and PHA (average r across four doses = 0.65; range = 0.60–0.69) and were averaged across sessions.

Natural killer cell cytotoxicity. NKCC was measured using a whole-blood assay (43) at effector-to-target cell ratios of 100:1, 50:1, 25:1, and 12.5:1. Cytotoxicity values obtained from the two GCRC sessions were moderately correlated ($r = 0.50$) and were averaged across sessions. Values are expressed in cytotoxicity units, which reflect aggregate killing across the effector-to-target cell ratios (43). Analyses are presented for raw cytotoxicity units and for adjusted cytotoxicity units (where natural killer cell counts have been statistically partialed out), so that we can assess whether depression-related alterations in NKCC were attributable to differences in per-cell killing vs. in-well cell counts.

Macrophage production of IL-1β. Production of the macrophage-derived proinflammatory cytokine IL-1β was assessed with solid-phase sandwich ELISA kits purchased from Biosource International (Camarillo, CA). Whole blood was diluted 1:5 in RPMI-1640 medium supplemented with 10 mM HEPES, 2 mM glutamine, and 50 μg of gentamicin. Five hundred microliters of diluted blood was added in duplicate to 24-well, flat-bottom plates containing 500 μl of lipopolysaccharide. After 48 hours of incubation at 37°C in a humidified 5% CO_2 incubator, supernatant was collected and stored at −70°C until assayed in one batch. The ELISA was performed according to the protocol of the manufacturer. Serum was diluted 1:16. A 96-well plate was then coated with IL-1β–specific antibody. Standards, controls, and unknown samples were added, followed by a biotinylated antibody directed against IL-1β and then streptavidin-peroxidase. The chromogen reaction was determined using tetramethyl benzidine and terminated with H_2SO_4. The colorimetric reaction was analyzed by using a plate spectrophotometer (Titertek Multiscan MSS/340) at 450 nm. IL-1β concentrations were extrapolated from a standard curve with linear regression from a log-linear curve.

Statistical Analyses

To examine whether depressed and control subjects differed with respect to immunologic parameters and whether these relationships were moderated by age, we performed between-subjects ANOVAs, with depression status (depressed vs. control subjects) and age (under vs. over 40 years of age) serving as independent variables. This yielded four cells containing younger depressed subjects (N = 18), older depressed subjects (N = 13), younger control subjects (N = 19), and older control subjects (N = 12). (Regression analyses treating age as a continuous variable result in the same conclusions). An analogous ANOVA strategy was used to determine whether the groups differed with respect to neuroendocrine parameters and/or health practices. To assess whether neuroendocrine parameters and/or health practices operated as pathways through which depression was associated with immunity, we followed Stone's recommendations for testing statistical mediation (44). According to this framework, three criteria must be met for data to be consistent with a mediational model. First, the predictor variable (in this case depression status) must be associated with the outcome variable (in this case an immune parameter). Second, the predictor variable must be associated with the proposed mediator (neuroendocrine parameters or health practices). Finally, the magnitude of the association between the predictor variable and the outcome variable must be substantially reduced when the proposed mediator is statistically controlled. We should note at the outset that these analyses do not provide a test of whether a proposed mediator acts in a causal manner. That would require an experimental manipulation of the mediator of interest. What they can provide, however, is an indication of whether the proposed mediator relates to depression and immunity in a way that is consistent with a mediational hypothesis.

RESULTS

Table 1 presents the demographic and clinical characteristics of the sample. Depressed and control subjects did not differ with respect to age, ethnicity, or years of education ($t(60) < 1.00$, NS). However, they were more likely than control subjects to be single and divorced or separated ($\chi^2(2) = 12.66$, $p < .01$). Depressed subjects reported higher levels of depressive symptomatology than controls on the BDI ($t(60) = 14.56$, $p < .001$) and more intense depressed affect on the POMS Depression Scale ($t(60) = 8.05$, $p < .001$). Overall, depressed subjects scores on the HAM-D and the BDI suggested that they were suffering from depressive episodes of mild to moderate severity.

Depression Status, Age, and Immune Parameters

Table 2 presents average circulating leukocyte subset counts for depressed and control subjects. As the table illustrates, the groups did not differ with respect to any of the cell phenotypes ($F(1,61) < 1.33$, $p > .25$). Main effects of age emerged for $CD8^+$ T lymphocytes and B cells, with younger subjects showing lower numbers of these cells ($CD8^+$ cells, $F(1,61) = 5.22$, $p <$

TABLE 1 Demographic and Clinical Characteristics of Sample[a]

	Depressed Group			Control Group		
	Mean	SEM	Range	Mean	SEM	Range
Demographic characteristics						
Age (years)	34.5	1.4	22–48	34.4	1.5	20–48
Education (years)	15.2	0.4	8–22	16.9	0.7	10–22
Race/ethnicity (%)						
White	87.5			87.5		
African American	12.5			12.5		
Marital status (%)						
Married*	31.3			56.3		
Single*	50.0			37.5		
Separated or divorced*	18.8			6.3		
Clinical characteristics						
HAM-D	15.7	0.8	8–26			
BDI**	24.7	1.6	14–36	1.1	0.3	0–6
POMS**	19.7	2.4	1–46	0.6	0.2	0–3.50
Duration of current episode (months)	7.7	1.1	1–22			
Number of previous episodes	5.9	0.7	0–20			

[a] $N = 31$ for depressed group and $N = 31$ for control group. * depressed and control groups differ at $p < .01$, ** $p < .001$

TABLE 2. Circulating Leukocyte Subset Counts for Depressed and Control Subjects[a]

Leukocyte[b]	Depressed Group		Control Group	
	Mean	SEM	Mean	SEM
White blood cells	6570.0	325.1	6160.0	265.8
Granulocytes	4200.0	269.4	3800.0	222.7
Monocytes	480.0	32.3	450.0	19.8
Lymphocytes	1630.0	66.5	1710.0	91.6
T lymphocytes ($CD3^+$)	1219.5	54.9	1297.8	74.7
Helper/inducer T lymphocytes ($CD3^+/CD4^+$)	803.0	40.8	822.0	55.1
Suppresser/cytotoxic T lymphocytes ($CD3^+/CD8^+$)	434.5	26.8	465.1	29.6
Natural killer cells ($CD3^-/CD56^+$)	161.7	12.3	147.4	9.5
B cells ($CD19^+$)	220.5	14.9	255.0	22.6

[a] $N = 31$ for depressed group and $N = 31$ for control group. No significant group differences emerged with respect to circulating leukocyte subset counts.
[b] Cells per cubic millimeter of peripheral blood (surface markers).

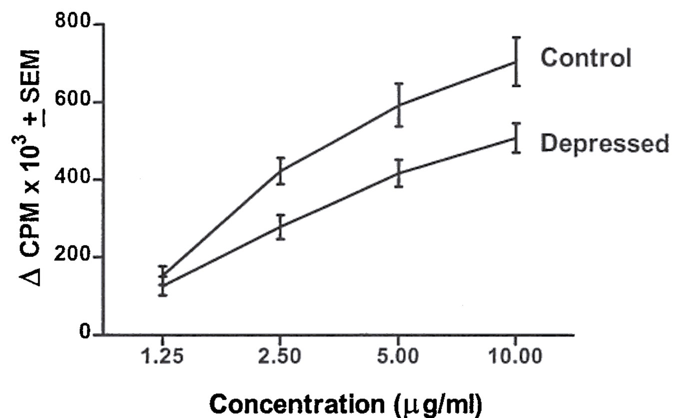

Fig. 1. Lymphocyte proliferation to ConA by depression status.

.03; B cells, $F(1,61) = 6.72$, $p < .02$). None of the other cell phenotypes differed by age. There were no statistically significant depression status-by-age interactions for circulating subset counts ($F(1,61) < 1.90$, $p > .17$). Leukocyte subset percentages also did not differ according to depression status, age, or the interaction of these variables ($F(1,61) < 1.00$, NS).

Figure 1 presents lymphocyte proliferative responses to ConA. These data were analyzed using a three-way mixed ANOVA with depression status and age as between-subjects factors and dose as a within-subjects factor. A significant main effect of depression status emerged, such that depressed subjects exhibited reduced proliferative responses to the mitogen across all four doses compared with control subjects ($F(1,58) = 4.38$, $p < .04$). This effect was qualified by a significant depression status-by-dose interaction ($F(3,174) = 2.76$, $p < .05$). Post hoc t tests indicated that depressed subjects had smaller responses to ConA at doses of 2.50 μg/ml ($t(60) = -2.11$, $p < .05$), 5 μg/ml ($t(60) = -2.25$, $p < .04$), and 10 μg/ml ($t(60) = -2.30$, $p < .04$). However, depressed and control subjects did not differ at the 1.25-μg/ml dose ($t(61) = -0.69$, NS). The main effect of age did not achieve statistical significance for ConA ($F(1,58) = 2.12$, $p > .15$), and neither did the depression status-by-age, dose-by-age, or depression status-by-age-by-dose interactions ($F < 1.00$, NS).

As Figure 2 illustrates, a main effect of depression status also emerged for PHA, such that depressed sub-

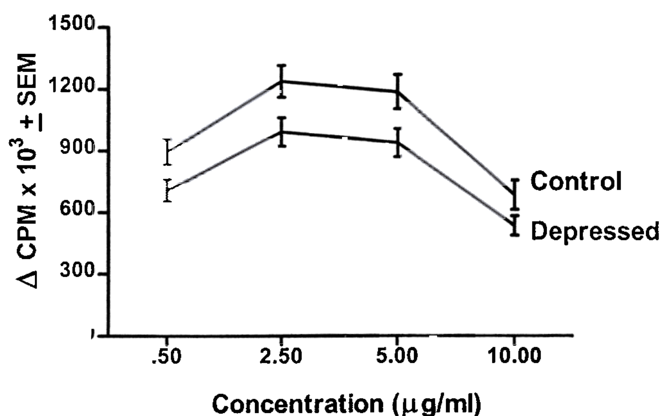

Fig. 2. Lymphocyte proliferation to PHA by depression status.

jects had smaller proliferative responses than control subjects across all four doses ($F(1,58) = 4.09$, $p < .05$). The depression status-by-dose interaction for PHA was not statistically significant ($F(3,174) < 1.00$, NS). PHA responses also did not vary as a function of age, and no statistically significant two- or three-way interactions emerged for this mitogen ($F < 1.00$, NS).

There were no differences in NKCC between depressed and control subjects or between older and younger subjects ($F(1,61) < 1.00$, NS). There was a significant depression status-by-age interaction for NKCC ($F(1,61) = 5.65$, $p < .03$). As Figure 3 illustrates, depression was accompanied by a relative enhancement in NKCC among younger subjects and a relative reduction among older subjects. Follow-up analyses using adjusted cytotoxicity units (where natural killer cell counts had been statistically controlled) yielded identical results to those described above, suggesting that age- and depression-related differences in NKCC were not simply a function of group differences in numbers of circulating natural killer cells.

With respect to the proinflammatory cytokine IL-1β, analyses revealed no difference between depressed and control subjects ($F(1,61) = 0.08$, NS). Mean values were 2750 (SEM = 229) for depressed subjects and 2840 (SEM = 195) for control subjects. Neither the main effect of age nor the depression status-by-age interaction was statistically significant for IL-β ($F(1,61) < 1.00$, NS).

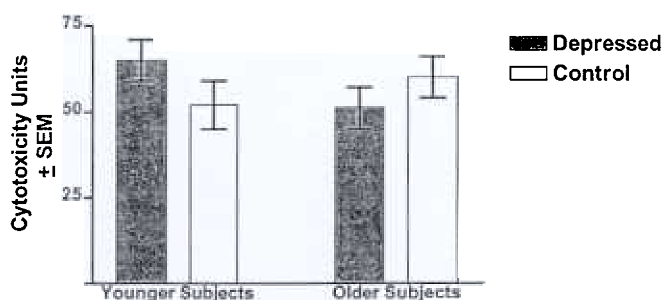

Fig. 3. NKCC by depression status and age.

Pearson correlations were computed to assess whether the accumulation of depressive symptoms was associated with more pronounced immunologic changes. Across the entire sample, higher scores on the BDI were associated with greater numbers of circulating white blood cells ($r(62) = 0.30$, $p < .04$) and granulocytes ($r(62) = 0.32$, $p < .03$). Additionally, subjects who reported more intense negative affect on the POMS exhibited greater numbers of natural killer cells ($r(62) = 0.25$, $p < .05$). When analyses were conducted separately for depressed and control subjects, both groups showed positive associations between BDI scores and white blood cell counts (depressed group, $r(31) = 0.42$, $p < .02$; control group, $r(31) = 0.37$, $p < .05$) and granulocyte counts (depressed group, $r(31) = 0.42$, $p < .02$; control group, $r(31) = 0.33$, $p < .08$). Within groups, no other significant associations emerged between severity measures (HAM-D, BDI, or POMS) and immune parameters ($p > .20$).

Depression Status, Age, and Potential Mediators

Means and standard deviations for neuroendocrine parameters are shown in Table 3. Depressed subjects had higher circulating levels of norepinephrine than control subjects ($F(1,61) = 4.06$, $p < .05$) and exhibited a trend toward higher estradiol levels ($F(1,61) = 3.32$, $p < .08$). No other group differences were observed ($F(1,61) < 1.24.$, $p > .27$). Main effects of age and depression status-by-age interactions did not achieve statistical significance for any of the neuroendocrine parameters ($F(1,61) < 3.10$, $p > .10$).

Table 4 presents descriptive statistics for health

TABLE 3. Neuroendocrine Parameters for Depressed and Control Subjects[a]

	Depressed Group		Control Group	
	Mean	SEM	Mean	SEM
Plasma hormones				
Cortisol (μg/dl)	13.1	1.0	13.5	1.1
Epinephrine (pg/ml)	28.7	2.5	30.3	3.0
Norepinephrine (log 10 pg/ml)*	2.4	0.1	2.3	0.1
Estradiol (pg/ml)**	92.9	9.7	70.8	8.3
Progesterone (nmol/l)	13.9	2.4	10.3	2.2
Urinary Hormones				
Free Cortisol (μg/total volume)	35.6	4.4	35.1	3.2
Total Cortisol (μg/dl)	2.9	0.3	2.8	0.2
Epinephrine (μg/total volume)	3.9	0.3	4.3	0.3
Norepinephrine (μg/total volume)	31.0	2.3	32.0	1.7

[a] $N = 31$ for depression group and $N = 31$ for control group. * Depressed and control groups differ at $p < .05$, ** $p < .08$.

TABLE 4. Health Practices Among Depressed and Control Subjects[a]

	Depressed Group		Control Group	
	Mean	SEM	Mean	SEM
Tobacco, alcohol, and caffeine consumption				
Current smokers (%)*	28.1		16.1	
Former smokers (%)*	25.0		6.5	
Alcoholic drinks/week	2.2	0.4	3.2	1.0
Caffeinated drinks/week*	19.0	1.4	14.0	1.6
Physical activity and body mass				
Physical activity index (0–6)**	2.0	0.3	3.7	0.3
Body mass index (kg/m^2)	26.1	1.2	25.9	0.9
Sleep parameters				
Sleep quality (1–4)**	2.7	0.1	3.5	0.1
Hours of sleep/night	7.1	0.3	7.1	0.2
Sleep efficiency*	0.9	0.1	0.9	0.1
Nutritional markers				
Albumin (mg/dl)	3813.8	49.4	3727.0	46.3
Transferrin (mg/dl)	252.8	8.9	249.1	8.5
Retinol-binding protein (mg/dl)	4.7	0.2	4.5	0.3

[a] $N = 31$ for depression group and $N = 31$ for control group. * Depressed and control groups differ at $p < .05$, ** $p < .001$.

practices. Depressed subjects were more likely to be current smokers and to have a history of smoking than control subjects ($\chi^2(2) = 6.80$, $p < .04$). They also reported less physical activity ($F(1,61) = 14.83$, $p < .001$), more caffeine consumption ($F(1,61) = 4.00$, $p < .05$), had poorer subjective sleep quality ($F(1,61) = 16.41$, $p < .001$), and had less efficient sleep ($F(1,61) = 4.80$, $p < .03$). Depressed and control subjects did not differ with respect to sleep duration, body mass index, alcohol consumption, or nutritional markers ($F(1,61) < 1.00$). There were no statistically significant main effects of age or depression status-by-age interactions for health practice variables ($F(1,61) < 2.70$, $p > .12$).

Pathways Linking Depression Status, Age, and NKCC

Next we explored potential explanations for the relative enhancement in NKCC among younger depressed subjects and relative decrement among older depressed subjects. One possibility is that this was due to differences in neuroendocrine regulation between younger and older depressed subjects and control subjects. However, as noted above, depression status-by-age interactions did not reach statistical significance for any of the neuroendocrine parameters. It is also possible that differences in health practices, body mass, or nutrition were responsible for this effect. However, statistically significant depression status-by-age interactions did not emerge for these variables either. Finally, it is possible that this pattern was attributable to younger and older depressed subjects having disparities in psychiatric history, episode severity, or episode duration. Although younger and older depressed subjects did not differ with respect to number of previous depressive episodes ($t(29) < 1.00$), younger subjects had marginally more severe symptoms on the HAM-D ($t(29) = 1.83$, $p < .08$) and had been depressed for a shorter period of time ($t(29) = 3.14$, $p < .01$). However, none of these variables was related to NKCC ($p > .30$). Overall, these analyses suggest that the disparity in NKCC between younger and older depressed subjects was not a result of differences in psychiatric symptoms or the neuroendocrine parameters or health practices assessed in this study.

Pathways Linking Depression Status and Lymphocyte Proliferation

The final set of analyses examined whether neuroendocrine parameters or health practices operated as pathways through which depression was associated with immunity. We focused here on identifying mediators of the relationship between depression and reduced lymphocyte proliferation, because this was the only immune parameter that differed reliably between depressed and control subjects. As noted above, the two neuroendocrine parameters that differed between depressed and controls subjects were norepinephrine and estradiol. However, neither of these hormones was associated with proliferative responses to ConA or PHA ($p > .40$). When depression status-by-age ANOVAs for ConA and PHA were recomputed with statistical control of norepinephrine and estradiol levels, there was not an appreciable reduction in the magnitude of the relationship between depression status and lymphocyte proliferation to either ConA or PHA (<10% reduction in variance accounted for). Statistical controls for epinephrine, cortisol, and progesterone also did not attenuate the association between depression and proliferative responses (<15% reduction in variance accounted for). When considered within Stone's (44) framework for assessing mediation, these findings suggest that the neuroendocrine pathways assessed in this study were not responsible for the depression-related alterations in lymphocyte proliferation.

As we noted above, depressed and control subjects differed with respect to a variety of health practices, including caffeine consumption, subjective sleep quality, sleep efficiency, smoking history, and frequency of physical activity. Only two of these were related to lymphocyte proliferation. Subjects with a history of

smoking had smaller PHA responses than subjects who had never smoked ($t(60) = -1.99$, $p < .05$), but the groups did not differ with respect to ConA responses ($t(60) < 1.00$, NS). Less frequent physical activity was associated with smaller proliferative responses to ConA ($r(62) = 0.28$, $p < .03$) and marginally smaller responses to PHA ($r(62) = 0.24$, $p < .06$). To assess the extent to which these health practices might contribute to the relationship between depression and lymphocyte proliferation, depression status-by-age ANOVAs for ConA and PHA were recomputed, each time including a health practice variable as a covariate. Partialing out smoking status, caffeine use, sleep quality, and sleep efficiency did not appreciably reduce the magnitude of the relationship between depression and either ConA or PHA responses (<10% decrease in variance accounted for in each case). Neither did partialing out the remaining health practice variables (ie, controls for sleep duration, body mass index, alcohol consumption, and nutritional markers resulted in <8% decrease in variance accounted for). However, partialing out physical activity substantially reduced the magnitude of the association between depression and proliferative responses. In the case of ConA, including physical activity as a covariate reduced the amount of variance that depression accounted for from 7.84% to 2.89%. This represents a 63% decrease [1 − (2.89/7.84)]. For PHA, partialing out physical activity reduced the amount of variance that depression accounted for by 42%, from 6.25% to 3.61% [1 − (3.61/6.25)]. Although causal inference is not possible here, these results are consistent with the hypothesis that physical activity operates as a mediator of the association between depression and immunity.

DISCUSSION

We found that ambulatory depressed patients exhibit reduced lymphocyte proliferative responses to the mitogens ConA and PHA compared with healthy control subjects. These findings are consistent with studies of severely depressed hospitalized patients (1, 45). Moreover, they may help to clarify some of the inconsistencies that have emerged in previous studies of ambulatory patients. We may have been able to detect differences in lymphocyte proliferation because of the high degree of diagnostic homogeneity within the depressed and control groups or because of the increased statistical power associated with averaging across two assessments of immune parameters, which substantially reduces the measurement error involved in in vitro immune assays with peripheral blood lymphocytes. Overall, this study provides additional evidence that depression-related immune changes are not restricted to severely depressed individuals undergoing treatment in an inpatient context (8, 10–14).

We found some evidence that the accumulation of depressive symptoms was associated with more pronounced immunologic change. Subjects with more symptoms on the BDI tended to have greater numbers of circulating white blood cells and granulocytes; however, symptom severity was unrelated to other enumerative and functional immune parameters. Previous studies of ambulatory patients also have failed to document extensive severity effects (8, 11, 13). This may be a result of the fairly restricted range of severity scores encountered in ambulatory settings. It is also conceivable that the magnitude of depression-related immune change is dependent on the intensity of negative affect rather than accumulation of symptoms (1). Consistent with this hypothesis, we found that subjects with more intense depressed affect on the POMS exhibited higher circulating numbers of natural killer cells.

Depression was associated with different patterns of NKCC in younger and older subjects. Younger subjects who were depressed exhibited a relative enhancement in NKCC, whereas older depressed subjects showed reduced NKCC. These findings are consistent with previous research showing that depression is accompanied by an enhancement in specific immunologic functions among younger individuals and a suppression of those functions among older individuals (4, 8, 13). However, whereas previous research has generally found this pattern of age-related differences with lymphocyte proliferative responses, we found it with natural killer cell cytotoxicity. It is possible that between-study differences in sample composition (eg, the inclusion of depressed patients with comorbid anxiety disorders or control subjects with a psychiatric history in earlier studies) are responsible for this disparity. More generally, it is not clear what mechanisms are responsible for immunologic differences between younger and older depressed patients. However, we were able to rule out a number of candidates in the present study, including severity, duration, and history of clinical depression, health practices, and the specified neuroendocrine parameters.

We also examined pathways by which clinical depression might result in alterations in immune parameters. Although depressed subjects exhibited higher circulating levels of norepinephrine and estradiol than control subjects, neither of these hormones was related to lymphocyte proliferative responses. Depressed and control subjects did not differ with respect to plasma levels of cortisol, epinephrine, or progesterone or urinary levels of cortisol, epinephrine, or norepinephrine. These findings suggest that neuroendocrine path-

ways are not responsible for the reduced lymphocyte proliferation among depressed subjects in this study. However, it is possible that measurement error attenuated the relationship between neuroendocrine and immunologic parameters (especially in the case of norepinephrine). Moreover, several neuroendocrine parameters (ACTH, β-endorphin, and thyroid hormones) that relate to both clinical depression and immunity could be involved in this process and were not assessed here (12, 18–20, 46). Finally, our findings do not rule out the possibility that neuroendocrine dysregulation is responsible for the alterations in cell-mediated immunity that have been observed in more severely depressed populations. Indeed, severely depressed individuals are more likely to manifest such neuroendocrine abnormalities (18, 19).

With respect to health practices, depressed subjects exhibited higher rates of tobacco and caffeine consumption, performed less physical activity, and had poorer sleep quality. Depression was not associated with differences in alcohol consumption, body mass index, or nutrition. Physical activity showed a consistent relationship with depression-related immune parameters, such that subjects with less frequent activity exhibited smaller proliferative responses to ConA and PHA. When frequency of physical activity was statistically controlled, the magnitude of depression's relationship with proliferation was reduced by 63% for ConA and 42% for PHA. These findings are consistent with physical activity acting as a pathway through which depression reduces lymphocyte proliferation. To our knowledge, these are the first published data in support of a mediational pathway that can account for immunologic differences *between* depressed and nondepressed individuals. Other studies have shown associations between immunity and such parameters as sleep continuity and psychomotor retardation (25, 32, 47). However, they have not evaluated whether these parameters mediate the relationship between depression and immunity. It is important to note that although our findings are consistent with a mediational model, they are not causally demonstrative of mediation. Such a demonstration would require manipulation of the putative mediator.

The cross-sectional design of this study precludes causal inferences about the relationship between depression and immunity. Given that the central nervous system and the immune system communicate along a bidirectional axis, it is conceivable that immunologic processes are the cause, rather than the consequence, of depression (30, 48). It also is possible that the relationship between depression and immunity stems from their common association with a third variable, such as physical activity. The lack of subjects older than 48 years may have hampered our ability to detect interrelationships between age, depression, and immunity. It was necessary to exclude menopausal and postmenopausal women from participation, however, so that we could assess the extent to which ovarian hormones contributed to depression-immunity relations. The lack of differentiation between subtypes of clinical depression (eg, typical vs. atypical presentation, melancholic vs. nonmelancholic presentation) may have obscured immunologic differences between groups. There is evidence that depressive subtypes may differ with respect to some immune parameters (10–12, 30). Finally, it is not clear what implications in vitro immunologic assays have for in vivo immune function.

This study demonstrates that depressed women exhibit reduced mitogen-stimulated lymphocyte proliferative responses compared with control subjects. It also demonstrates that clinical depression is accompanied by enhanced NKCC in younger women and reduced NKCC in older women. Although these findings provide additional evidence of a link between depression and altered immune response, perhaps the most important contribution of this study is that it identifies a pathway through which depression may become associated with decrements in lymphocyte proliferative response. It will be important for future studies to determine whether physical activity acts in a causal fashion. This might be accomplished by investigating whether interventions aimed at increasing physical activity can buffer people from the immunologic changes associated with depression. Exercise serves this stress-buffering function in the rat immune response (33). It also will be important for future research to determine the health implications of the immunologic alterations described here and in previous studies. It is conceivable that such alterations could help to explain the excess morbidity and mortality that has been repeatedly observed among depressed individuals (49–53).

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