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Depression, Urinary Free Cortisol Excretion and Lymphocyte Function

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An impairment in lymphocyte response to mitogen stimulation, a correlate of cell-mediated immunity, has been reported in patients with depressive illness. To investigate whether such impairment in lymphocyte function is related to excessive secretion of cortisol, an immunosuppressive hormone, we compared mitogen-induced lymphocyte proliferation in three groups of subjects: (1) depressed patients with elevated 24-hour urinary free cortisol (UFC) excretion; (2) depressed patients with normal UFC excretion; and (3) normal controls. Depressed patients in both groups showed significant reductions in lymphocyte mitogenic activity, in comparison with the normal controls, but the two depressive groups did not significantly differ from each other in their lymphocytic responses to any of the mitogens used. Furthermore, no significant correlations were found, within depressed patients, between UFC excretion and lymphocyte mitogenic responses. Depression is therefore associated with an impairment in lymphocyte function that cannot be explained solely on the basis of increased cortisol secretion.

The immune system is closely associated with both the central nervous system and the endocrine system (Rogers *et al*, 1979; Riley, 1981); recent developments in the psychobiology of the affective disorders suggest an increase in hypothalamic-pituitary-adrenal (HPA) activity in some but not all patients with depression (Carroll, 1982). This is usually manifested by increases in cortisol levels in plasma, CSF, and urine of depressed patients, compared to normal controls (Carroll *et al*, 1976a, 1976b). Other HPA abnormalities in depression include a disruption of the circadian pattern of cortisol secretion (Sachar *et al*, 1973) and plasma cortisol resistance to suppression by dexamethasone (Carroll, 1982). Physiologically, this increase in HPA activity in depression may result in a state of hypercortisolaemia, of varying intensity and duration. Since one major effect of hypercortisolaemia is suppression of immune function (Dougherty *et al*, 1964; Claman, 1972), and since impaired immunity has been observed in depressed patients (Kronfol *et al*, 1983; Schleifer *et al*, 1984), we postulated that depressed patients with hypercortisolaemia will have lower immunological competence than depressed patients without this. To test this hypothesis, we compared immune function in depressed patients with and without hypercortisolaemia and normal controls. The immunological parameter studied was lymphocyte response to mitogen stimulation—a correlate of cell-mediated immunity (Oppenheim *et al*, 1975). HPA activity was assessed with measures of 24-hour urine free cortisol, which is a sensitive indicator of adrenocortical function (Beardwell *et al*, 1968; Tyler & West, 1972) and a good index of circulating unbound and physiologically active plasma cortisol levels over time (Burke & Beardwell, 1973; Carroll *et al*, 1976c). We found that depressed patients had lower lymphocytic responses than normal controls, but that this lowering of immunological competence in depression was not related to increased levels of UFC excretion.

Method

Subjects

Three groups of subjects were identified: (1) depressed patients with hypercortisolaemia ($n = 11$); (2) depressed patients without hypercortisolaemia ($n = 22$); and (3) normal controls ($n = 20$). Hypercortisolaemia was defined, on the basis of UFC excretion, as levels exceeding 100 μg/24 hours; this was based on published reports on UFC determinations using similar techniques and showing mean UFC values for the normal population in the range of 33–48 μg/24 hours, with less than 5% of the normal population having values in excess of 100 μg/24 hrs (Murphy, 1968; Beardwell *et al*, 1968; Hsu & Bledsoe, 1970). The 100 μg/24 hours criterion has also been used by our group to differentiate between depressed and non-depressed psychiatric patients (Carroll *et al* 1976b; Carroll *et al*, 1976c).

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All patients were evaluated at the Clinical Studies Unit of the Department of Psychiatry, University of Michigan Medical Center; they all met the Research Diagnostic Criteria (Spitzer *et al*, 1977) for major depressive disorder. Controls were healthy volunteers with no history of psychiatric illness. Demographic features of the three study groups are presented in Table I. Depressed patients

TABLE I
Demographic features

Diagnosis (n)	Age	Sex (M:F)	HDRS
Depression with elevated UFC (11)	48.8±18.1	4:7	19.7±5.5
Depression with normal UFC (22)	42.0±18.2	7:15	15.3±6.3
Normal Controls (20)	36.7±14.9	8:12	—
P values	NS	NS	NS

with elevated UFC tended to be older than the other two groups, but these differences were not statistically significant ($F = 1.83$; $df = 2,50$; NS). Similarly, there were no significant differences in the sex distribution of the three groups ($X^2 = 0.83$; $df = 2$; NS). Severity of depression, as reflected by scores on the Hamilton Depression Rating Scale (HDRS—Hamilton, 1960), was not significantly different between the two depressive groups ($t = 1.9$; $df = 31$, NS). All patients and controls were in good physical health and were drug-free for at least two weeks prior to the immune evaluation. Informed consent was obtained from all participants.

Procedure

1. UFC. Urine collections for 24-hour UFC determinations were started on Day 1 at 2300 hours and completed on Day 2 at 2300 hours. Urine volumes were recorded and UFC levels measured using a modified version of the competitive protein-binding assay (Murphy, 1968; Carroll, 1976b). Mean urinary volume in the depressed patients with hypercortisolaemia was 1287±651 ml. Mean UFC value in these patients was 147.0±34.4 μg/24 hour. Mean urine volume in depressed patients without hypercortisolaemia was 1086±598 ml. Mean UFC was 60.3±20.3 μg/24 hours. The difference in urine volume between these two groups was not statistically significant ($t = 0.88$; $df = 31$; NS). The difference in 24-hour UFC was highly significant ($t = 9.13$; $df = 31$; $P < .0001$).

2. Lymphocyte response to mitogen stimulation. Twenty milliliters of blood were collected from each subject at 0800 hour on Day 2 of the study; all samples were thus drawn during the same 24-hour period of the urine collection. The blood was centrifuged over Ficoll-Hypaque and the lymphocytes separated and washed; lymphocytes were then re-suspended in a culture medium (Kronfol *et al*, 1983). Triplicate lymphocyte cultures were established in microtiter plates. To each well were added 10^5 lymphocytes from the culture medium and one of the following mitogens: Phytohemagglutinin-P (PHA), 25 μg/ml, Concanavalin A (Con A), 10 μg/ml or Pokeweed Mitogen (PWM), 1:10 dilution. These doses of mitogen were used because they were associated with optimal stimulation in dose-response curves obtained earlier in our laboratory. Lymphocytes from all subjects were also cultured without mitogen for background stimulation. All cultures were incubated at 37°C for 96 hours—a culture period determined in our laboratory to provide optimal results; at this point, 1 μCi of tritiated thymidine was added to each well, and six hours later, the cultures were harvested on to glassfibre filter paper, washed, and air-dried. Tritiated thymidine uptake, indicative of the mitotic activity of the lymphocytes, was measured with a scintillation counter. The arithmetic means of the triplicate samples for each subject were then calculated. The lymphocyte response to each mitogen was expressed as the difference in scintillation counts per minute (cpm), between mitogen and background counts.

3. Statistical analysis. The arithmetic mean (±SD) of the lymphocyte response to each mitogen in each group was calculated and recorded. For statistical comparisons between the groups we used the student t test. For correlations between the endocrine and immune variables, we calculated Pearson's correlation coefficient r. All levels of significance were two-tailed.

Results

The background (unstimulated) counts for the three study groups were as follows: 346±176 cpm for depressed patients with elevated UFC; 835±480 cpm for depressed patients with normal UFC and 558±288 cpm for normal controls. The lymphocytic responses to the mitogens PHA, Con A, and PWM in the three groups were calculated as described above and illustrated in Figure 1. Depressed patients with elevated UFC had significantly lower lymphocytic responses to the mitogens PHA ($t = 3.43$; $df = 29$; $p = .0018$), Con A ($t = 2.45$; $df = 29$; $P = .0207$) and PWM ($t = 3.27$; $df = 29$; $P = .0028$) than the

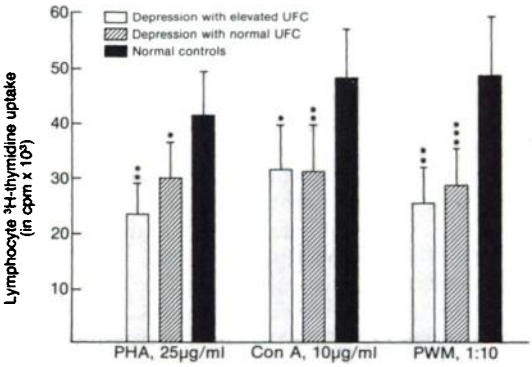


FIG. 1 Lymphocyte response to mitogen stimulation in depressed patients with and without hypercortisolaemia and normal controls. Comparisons are made between each of the depressed group and the normal controls.
*$P < .05$ **$P < .01$ ***$P < .001$

normal control group. Depressed patients with normal UFC also had significant reductions in their lymphocytic responses to PHA ($t = 2.66$; $df = 40$; $P = .0113$), Con A

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($t = 3.18$; $df = 40$; $P = .0028$) and PWM ($t = 3.62$; $df = 40$; $P = .0008$) when compared to the normal controls. However, when depressed patients with elevated UFC were compared to depressed patients with normal UFC, no significant differences emerged. Lymphocyte responses to PHA ($t = 1.42$; $df = 31$; NS), Con A ($t = 0.10$; $df = 30$; NS) and PWM ($t = 0.67$; $df = 31$; NS) were not significantly different in these two groups.

We then examined the relation between UFC and lymphocyte function for the entire depressed group ($n = 33$). Table II shows the results of correlations between 24-hour UFC values and lymphocytic responses to the three different mitogens. There were no significant associations between UFC and lymphocytic responses to any of the mitogens used.

TABLE II

Correlations between UFC and lymphocyte response to mitogen stimulation in all depressed patients

Mitogen	*Pearson's r*	*n*	*P*	*Significance*
Phytohemagglutinin-P	−.22	33	.23	NS
Concanavalin A	+.0009	33	.99	NS
Pokeweed mitogen	−.06	33	.69	NS

Discussion

We have earlier reported a suppression of mitogen-induced lymphocyte proliferation in patients with depressive illness (Kronfol *et al*, 1982; Kronfol *et al*, 1983; Kronfol & House, 1984). These findings have also been replicated by independent investigators (Schleifer *et al*, 1984). However, none of the studies published so far has compared lymphocyte function in neuroendocrine sub-types of depression and normal controls. Our data indicate that depressed patients, regardless of cortisol secretion, have lower *in-vitro* lymphocyte mitogenic responses than normal controls. Lymphocyte response to mitogen stimulation is known to be affected by a number of factors such as age, diurnal variation, medical illness, and drugs. There were no significant differences in the age or sex distribution of our depressed patients and normal controls. All subjects were free of drugs or medical illness known to interfere with lymphocyte proliferation *in-vitro*. All blood samples were collected at the same time of the day to minimize possible effects of diurnal variation. Severe malnutrition has also been associated with immunosuppression, but our depressed patients were not malnourished (mean weight: 66.4 kgs). It is possible that the stress of hospitalisation may affect immune function, but since most of our patients had been in the hospital for a two-week drug washout period prior to immune testing, the stress of acute hospitalisation was unlikely to have played a major role.

Our findings also indicate, rather surprisingly, similar lymphocytic responses in depressed patients, regardless of the presence of hypercortisolaemia. Since elevated cortisol levels are usually associated with immunosuppression (Claman, 1972; Britton *et al*, 1975), we had expected depressed patients with elevated UFC to have lower lymphocytic responses than depressed patients with normal UFC, but our data did not support this hypothesis. We also failed to establish any significant correlation among depressed patients between UFC excretion and lymphocytic responses. Although it is not clear at what level of cortisol the lymphocyte function becomes disturbed, it is possible that the hypercortisolaemia observed in our depressed patients may not be severe enough to affect the immune response (Fauci, 1978). It is also possible that depression with hypercortisolaemia is associated with a state of glucocorticoid resistance which minimizes the physiological effects of cortisol (Schlecte & Sherman, 1985). Alternatively, it is possible that the immunosuppression in depressed patients may not be related to cortisol, but to other hormones or neurotransmitters which have been postulated to play a role in the pathophysiology of depression, such as catecholamines, serotonin, acetylcholine, and endorphins (Van Praag, 1982). Since all of these substances have also been shown to regulate the immune response (Hall & Goldstein, 1981), it is possible that a derangement in the metabolism of one or more of these substances in depression may be responsible for the observed immunological aberrations in depressed patients (Kronfol, 1985; Kronfol & Schlechte, 1985).

Thus, diminished parameters of immune function have been observed in patients with depressive illness. The present data indicate that this suppression of immunological competence is not related to an increase in levels of cortisol, an immunosuppressive hormone. We are presently studying the relationship between lymphocyte function and other biological and clinical variables in depressive illness. Further research is needed into the 'psychoneuro-immunology' of the affective disorders and into the relationship betwen hormones, mood, and the immune response in depressed patients to clarify some of these complex issues.

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