FUTURE DIRECTIONS FOR BRAIN, BEHAVIOR, AND THE IMMUNE SYSTEM*

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THE NOTION THAT STRESS MAY INFLUENCE disorders attributable to alterations in the immune system is a widely held popular belief. Woody Allen, for example, in the movie *Manhattan* said, "I can't express anger. That's one of the problems I have; I grow a tumor instead." Public interest has even extended to the belief that stress can affect pet birds and cause significant changes in the immune system. Is the interaction among brain, behavior, and the immune system "for the birds" or is there a body of scientific facts that indicates that this is a promising area for future research? Over the past decade we have witnessed an explosive growth in neurobiological and immunobiological research. The investigation of the relationship among the central nervous system, behavior, and the immune system has undergone a parallel period of rapid expansion with compelling evidence for central nervous system and behavioral interactions with the immune system. It is not clear at this time, however, if these interactions have clinical relevance.

The evolution of my own research over more than 40 years has included a consideration of brain, behavior, and the immune system. Some examples of research from my laboratory will be presented as a way to consider some of the issues that may be involved in determining future directions for this area of investigation.

My initial interest in the lymphocyte in the mid- to late 1940s stemmed from observations that there was hyperplasia of lymphoid tissue in patients suffering from Addison's Disease[1] and that adrenalectomy in experimental animals produced lymphoid tissue hyperplasia.[2] Dougherty and White[3] in 1945 suggested, based upon their histological examination of lymphoid tissue following adrenalectomy or administration of adrenotropic hormone in mice, that activation of pituitary-adrenal cortical secretion produced "accidental involution" of lymphoid tissue. The degeneration of lymphocytes was pro-

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* Presented as the Fifty-Eighth Thomas William Salmon Lecture of the Salmon Committee on Psychiatry and Mental Hygiene of the New York Academy of Medicine December 7, 1989.

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posed to be the mechanism by which lymphocytes contributed to gamma globulin in the blood and antibody protein in immunized animals. Based upon these observations, Long[4] utilized circulating blood lymphocyte levels as a way to study adrenal cortical activity. The number of circulating blood lymphocytes was employed in a number of studies to determine if the pituitary-adrenal cortical system was intact in schizophrenic patients. Pincus, Hoagland, and coworkers[5] failed to find in schizophrenics the lymphocytopenia they had observed following stress in normal individuals and concluded that the pituitary-adrenal system was defective in schizophrenic patients. A study in the laboratory in which I was working as a medical student at Washington University, however, found no differences in the blood lymphocyte counts of schizophrenic patients before and after electroshock, nor in the number of lymphocytes of schizophrenic patients and healthy controls following the injection of epinephrine.[6] Pincus and Hoagland[7] objected that electroshock was too gross a stress and that epinephrine effects were not comparable to the other forms of stress that had been used. I extended this research and investigated the physiological responses to heat stress and the injection of ACTH in normal and schizophrenic subjects, and found lymphocytopenia in both groups.[8] The number of circulating eosinophils was also measured since they provided a more reliable measure than the utilization of an absolute lymphocyte count;[9] all subjects showed a significant decrease in the eosinophil response. At this time and for the next few years, my research interests turned to preclinical studies, and, specifically, hypothalamic-pituitary interrelationships and the sequence of physiological events involved in the secretion of ACTH and other hormones of the anterior pituitary.[10]

In the mid 1950s my research was directed to the psychophysiology of bronchial asthma. A wide range of mechanisms appear to be involved in bronchial asthma, and psychosocial factors may play a role by modifying the immunologic processes that may be involved in some cases. Many experimental studies demonstrate the effect of psychosocial processes on immunologic responses, and these have been reviewed in detail.[11] The processes that might mediate psychosocial influences on immune function are complex and need further clarification.

In 1958 Freedman and Fenichel[12] reported that bilateral midbrain lesions in the guinea pig inhibited lethal anaphylactic death. Following that report, my attention was directed to the effect of lesions of the hypothalamus on anaphylaxis. The investigation of the relationship of the hypothalamus to immune function seemed appropriate since the hypothalamus is involved in the regulation of endocrine and neurotransmitter processes. Both of these

systems participate in the modulation of humoral and cell-mediated immunity. Szentivanyi and Filipp[13] were among the first to study the role of the hypothalamus in anaphylaxis. They demonstrated that lethal anaphylactic shock in guinea pigs and rabbits can be prevented by bilateral focal lesions in the tuberal regions of the hypothalamus. In our initial studies in the early 1960s we found that anterior but not posterior hypothalamic lesions inhibited the development of lethal anaphylaxis in rats.[14]

Further studies in our laboratory investigated the effect of hypothalamic lesions on guinea pig anaphylaxis.[15] Investigation of guinea pig anaphylaxis is relevant to an understanding of bronchial asthma, since the shock organ is the lung, and the animals die from massive bronchoconstriction.[16] We found that highly significant protection was afforded against lethal anaphylaxis in guinea pigs with electrolytic lesions in the anterior basal hypothalamus. Lethal anaphylaxis occurred in 71% of control animals and only in 18% of the guinea pigs with anterior hypothalamic lesions. Median and posterior hypothalamic lesions had no effect.

The effects of hypothalamic lesions on anaphylaxis could be explained both by antigen specific and nonspecific changes in the immune system as well as by changes in tissue factors and target organ responsivity.[17] A series of studies were undertaken to clarify some of the mechanisms that may be involved in the protective effect of anterior hypothalamic lesions. The findings suggested that the hypothalamic effect appears to be related to immune components of the anaphylactic reaction. While the hypothalamus may influence the target organ, i.e., the lung, the effects are not sufficient to inhibit anaphylaxis.

In view of these findings and others, the direction of research in our program became increasingly concerned with brain and behavior and the immune system. In keeping with my clinical interests, my research began to focus on the effects of stress on the immune system. A variety of stressors have been found to alter both humoral and cell-mediated immunity.[11] The specific nature and intensity of the stimulus as well as the biologic, psychologic, and social characteristics of the organism appear to be involved in the immune response to stress.

Conjugal bereavement is among the most potentially stressful of commonly occurring life events, and has been associated with increased medical mortality. The most compelling data regarding bereavement and health are derived from epidemiologic studies, and a definitive study has been conducted by Helsing and associates[18] in a prospective epidemiologic investigation of conjugal bereavement in approximately 4,000 subjects. They found the relative risk of death among widowers, especially between the ages of 55 and 74 years,

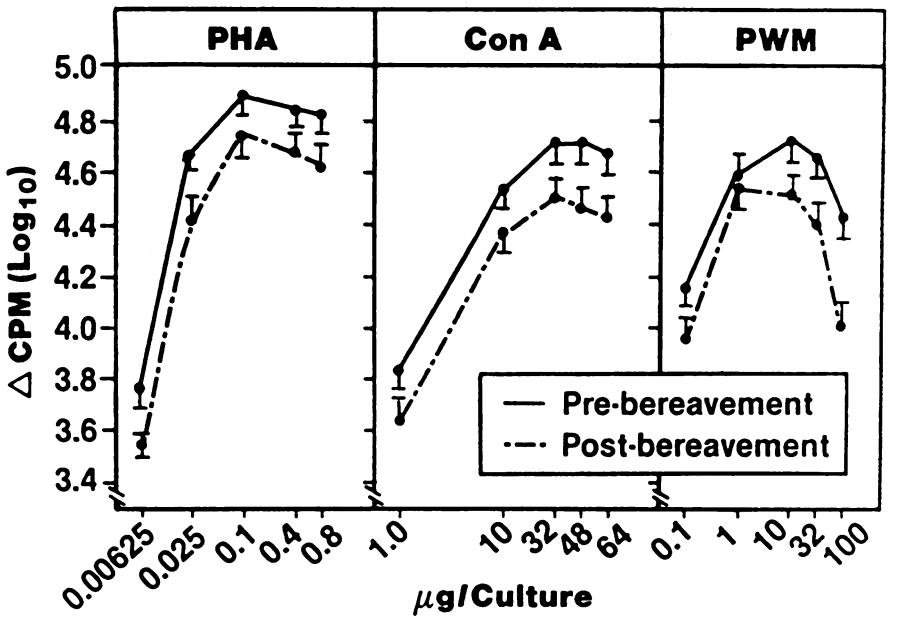

Fig. 1. Mitogen-induced lymphocyte stimulation before and after (1–2 months) bereavement. Each point represents group mean ± SEM of each subject's mean logΔCPM for each period. Reproduced by permission from Schliefer, S.J., Keller, S.E., Camerino, M., Thornton, J.C., and Stein, M.: Suppression of lymphocyte stimulation following bereavement. *J.A.M.A. 250:* 374–77, 1983.

significantly greater than among their married matched controls. A link between bereavement and altered measures of the immune system was suggested by Bartrop and coworkers,[19] who found that bereaved individuals had lower mitogen-stimulated lymphocyte proliferative responses compared to controls.

Our laboratory investigated the effects of bereavement on immune measures in a prospective longitudinal study of spouses of women with advanced breast carcinoma.[20] Lymphocyte stimulation was measured in 15 men before and after the deaths of their wives. Lymphocyte stimulation responses to the mitogens phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM) were significantly lower during the first two months following bereavement compared with prebereavement responses (Figure 1). The number of peripheral blood lymphocytes and the percentage and absolute number of T and B cells during the prebereavement period did not significantly differ from those in the postbereavement period. Follow-up during the remainder of the postbereavement year revealed that lymphocyte stimulation responses had returned to prebereavement levels for most but not all of the subjects.

Moreover, prebereavement mitogen responses did not differ from those of age- and sex-matched controls. These findings demonstrate that suppression of mitogen-induced lymphocyte stimulation is a direct consequence of the bereavement event and that a pre-existing suppressed immune state does not account for the depressed lymphocyte responses in the bereaved. Furthermore, the long-term stress of the spouse's illness does not appear to result in habituation of the lymphocyte's response to stress following bereavement. The long-term stress may, in fact, have sensitized the subject to the effects of bereavement.

The processes linking the experience of bereavement to effects on lymphocyte activity are complex. Changes in nutrition, activity, exercise levels, sleep, and drug use, which are often found in the widowed, could influence lymphocyte function. Our subjects, however, did not report major or persistent changes in diet, activity, or the usage of medication, alcohol, tobacco, or other drugs. No significant changes in weight were noted. Further study is required to determine if subtle changes in these variables are related to the effects of bereavement on lymphocyte function.

It is of note that the pattern of decreased mitogen stimulation responses varied among our subjects following the death of their spouses. For some subjects, a significant decrease was found in response to only one or two of the mitogens. However, in a small number of these bereaved men, significant decreases in lymphocyte proliferative responses were observed following stimulation with all three of the mitogens. This overall lack of mitogen-induced proliferative response may be important because impaired immune responses have been associated with increased mortality rates in two prospective studies. In one study,[21] a group of ambulatory individuals older than 85 with minimal delayed-type hypersensitivity skin reactions had more than twice the mortality rate over a two-year period than a similar group with robust delayed-type hypersensitivity responses. A more recent study observed a significant association between lack of responses to the three mitogens PHA, ConA, and PWM, and increased mortality in a group of more than 400 healthy, elderly subjects.[22] These reports suggest a possible relationship between a lack of immune response and mortality.

Nevertheless, it is important to emphasize that the immune findings associated with bereavement do not adequately explain the epidemiologic findings of increased morbidity and mortality following bereavement. The causes of death following bereavement are primarily associated with the cardiovascular system and not the immune system.[23] It remains to be determined whether stress-induced immune changes, such as decreased mitogen

responses, are related to the onset or course of physical illness following life stress. A major consideration of future directions for the study of brain, behavior, and the immune system is the *clinical relevance* of altered immune measures associated with behavior and the brain.

The manner in which experience is translated into biological consequences is another important consideration in the relationship between bereavement and immune function. Stressful life experiences, such as spousal bereavement, may lead to changes in central nervous system activity associated with psychologic states such as depression. Bereaved subjects have been characteristically described as manifesting depressed mood,[24] and a subgroup of bereaved individuals has been reported to have symptom patterns consistent with the presence of major depressive disorder.[24]

Several studies suggest that the severity of depressive symptoms accompanying bereavement may be related to decreased mitogen-stimulated lymphocyte proliferative responses,[25] reduced natural killer cell activity, and decreased T suppressor cell numbers.[26,27] It should be noted, however, that these studies have not been replicated and are limited by small sample size and a range of confounding influences which pose threats to the validity of the observations. Nonetheless, it has recently been demonstrated that plasma adrenocorticotropic responses to infusions of corticotropin releasing hormone in individuals with bereavement complicated by a major depressive episode were similar to those of patients with a major depressive disorder.[28] This response pattern was not observed in bereavement uncomplicated by depression. It appears that, among subgroups of bereaved individuals with depression, there may be neurobiologic alterations that can influence immune responses.

Depression and the Immune System

A number of studies have considered the association of psychiatric disorders and, specifically, depression and increased medical morbidity or mortality. The causes of death among patients with psychiatric disorders have recently been shown not to be significantly associated with death from natural causes, but rather with unnatural mortality, such as suicide and accidental death.[29–32] In addition, the causes of death varied according to diagnostic category: no excess mortality among patients with primary affective disorder, excessive unnatural mortality among patients with secondary affective disorder, and significant excess mortality due to unnatural causes and circulatory system disease among patients with panic disorder.[33]

Despite the lack of substantial evidence supporting an association between

depression and increased morbidity or mortality due to disorders involving the immune system, a great deal of attention has been directed to the notion that depression may influence immunocompetence in such physical disorders as cancer, autoimmune disorders, and infections, including human immunodeficiency virus (HIV). In the past decade a growing number of reports have provided a large body of data on syndromal depressive disorders and the immune system; however, these studies have led to disagreement and confusion regarding conceptualization, methods, experimental design, and results.[34] Furthermore, the relevance of immune alterations, reported to be present in depressive disorders, to health and illness remains unclear.

In an effort to clarify the current state of research concerned with immune alterations in depression, I shall review both methodologic and conceptual issues that may be involved in the investigation of depressive disorders and the immune system. The first report that considered the immune system in depressive disorders was published in 1978.[35] Since that time, more than 25 articles have appeared in peer-reviewed journals.[34] Twenty-two of these studies included experimental groups with the diagnosis of major depression (MD)/major depressive disorder (MDD) and control groups of apparently healthy subjects. Several additional published studies have utilized ill subjects, e.g., schizophrenic patients, as controls, and are not included in this review. In addition, as far as could be ascertained, the 22 studies did not have overlapping data bases. The measures of the immune system in these reports included both the enumeration of immune cells and assays of immune cell function.

Numerical changes in the number of lymphocytes can reflect significant alterations in the immune system in relation to a variety of diseases.[36] The enumeration of lymphocytes and the quantification of subsets of the major lymphocyte populations may provide relevant and important information about the immune system in depressive disorders. Eleven of the 22 studies in this review included cellular enumeration.[34] As can be seen in Table I, eight of these reports evaluated the total number of lymphocytes, and the only studies to observe a difference between depressives and healthy controls were those by Schleifer and coworkers[37] and Kronfol and House[38] who reported a decrease, or lymphopenia, in the depressed patients. The fact that only two studies reported a MD/MDD-associated lymphopenia is somewhat surprising in that hypercortisolemia is a characteristic of MD/MDD,[39] and it is well known that corticosteroid administration[40] and, as previously noted, increased corticosteroid secretion[3,4] are associated with lymphopenia. Corticosteroid administration has also been associated with an increase in neutrophilic

TABLE I. SUMMARY OF STUDIES OF CELLULAR ENUMERATION IN DEPRESSIVE DISORDERS

Variable	Total	Number of studies ↓	→
Lymphocytes	8	2	6
Total T cells	9	2	7
T Helper cells	4	—	4
T suppressor cells	4	—	4
B cells	6	1	5
Natural killer cells	2	1	1

↓ indicates significant difference in response of patients vs controls.
→ indicates no difference between patients and controls.
Adapted by permission from Stein, M., Miller, A.H., and Trestman, R.L.: Depression, the immune system, and health. Findings in search of meaning. *Arch. Gen. Psych. 48:*171–77, 1991.

granulocytes or neutrophilia.[41] Five reports measured neutrophil numbers in MD/MDD,[34] and only one study of these five[42] described a neutrophilia. None of these studies reported a pattern of neutrophilia coupled with lymphopenia.[34]

It is important to note that biologically significant alterations in lymphocyte subsets may occur without changes in the total number of circulating lymphocytes.[43] These lymphocyte subsets, which include T cells, T-cell subsets, and B cells, are defined by their differential functions and specific cell-surface markers. Nine of the 22 studies measured the number T cells, and only Schleifer and colleagues[44] found a decrease in the number of T cells in a group of ambulatory patients with MDD (Table I). The T-cell subsets were examined in four studies, and only one study reported any alterations in the depressed group (Table I). Six studies assessed the number of B cells in MD/MDD, and only Schleifer and colleagues[37] observed a decrease in the number of B cells in a hospitalized group of patients with MDD (Table I).

Most studies investigating the number of immune cells in the peripheral blood of individuals with MD/MDD have reported no differences. A range of methodologic and experimental design factors may be confounding the enumeration and interpretation of cell numbers in depressed patients and will be discussed below. In general, however, this review indicates that alterations in the number of immune cells is not a characteristic of patients with MD/MDD.

Among the available laboratory assays of immune function, two that have received most attention in relation to MD/MDD are the lymphocyte proliferative response to mitogens and the natural killer (NK) cell assay. Lymphocyte proliferation has been widely used in the assessment of a variety of immunologic disorders and in the study of lymphocyte biology.[45] It is not

TABLE II. SUMMARY OF STUDIES OF ASSAYS OF IMMUNE FUNCTION

Variable	Total	Number of studies ↓	→	↑
Mitogen Stimulation:				
PHA	14	6	7	1
ConA	11	6	5	—
PWM	11	6	5	—
Natural Killer Cell Activity	7	5	2	—

↓ indicates significant difference in response of patients vs controls.
→ indicates no difference between patients and controls.
↑ indicates significant increase in response of patients vs controls.
Adapted by permission from Stein, M., Miller, A.H., and Trestman, R.L.: Depression, the immune system and health. Findings in search of meaning. *Arch. Gen. Psych. 48*:171–77, 1991.

surprising, therefore, that it has attracted much attention in the investigation of the immune system in depressive disorders. Fourteen of the 22 studies have examined lymphocyte responses to one or more of the mitogens PHA, ConA, and PWM (Table II).[34] Six of the studies found the mitogen-stimulated responses to be decreased compared with controls, seven reported no significant alterations in the lymphocyte proliferative responses of the depressed patients, and one study reported an increased PHA response.[34]

Natural killer cells are a lymphocyte subset involved in the recognition and destruction of malignant and virus-infected cells. Seven studies have examined NK cell activity in depressive disorders.[34] and five of these reports observed decreases in NK cell activity in patients with MD/MDD compared with healthy controls (Table II). Mohl and coworkers[46] and Schleifer in our laboratory,[47] however, found no significant differences.

As with the enumeration of lymphocytes in depressive disorders, no consistent or reproducible alterations of functional measures of lymphocytes, namely, lymphocyte proliferative response and NK cell activity, have been reported in MD/MDD patients. However, of all of the findings, alterations of NK cell activity appear to be the most promising. Although the detailed specificity of NK cell function has not been defined, the NK response may be more specific than a functional measure, such as mitogen stimulation, and of more clinical importance, since there is increasing evidence of the *in vivo* relevance of NK cells to health and illness.[48] The failure to confirm any functional alteration may be due to a number of potential confounds in the experimental designs and a variety of methodologic flaws.

A range of methodologic concerns limits the interpretation and generalizability of the majority of the cited studies. These issues include diagnostic

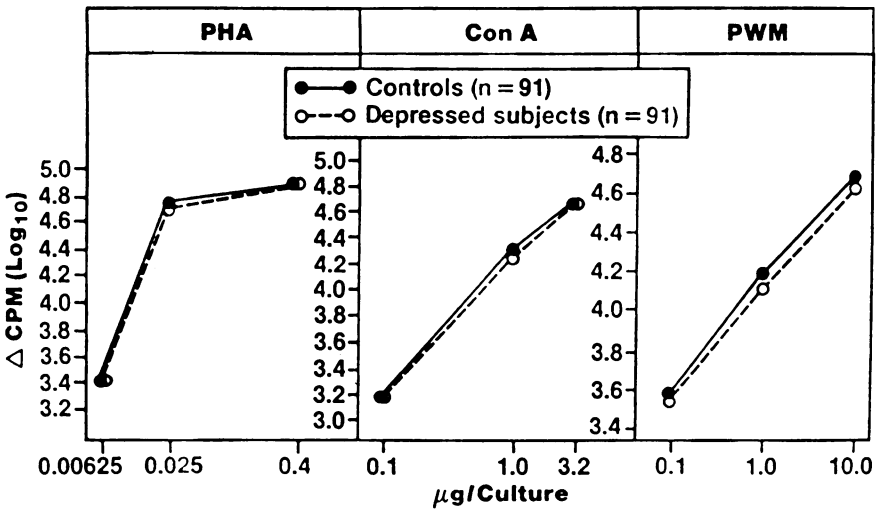

Fig. 2. Mitogen-induced lymphocyte stimulation in depressed patients and controls. Each point represents the mean ± SEM (logΔCPM). Reproduced by permission from Schiefer, S.J., Kelly, S.E., Bond, R.N., et al.: Major depressive disorder: role of age, sex, severity, and hospitalization. *Arch. Gen. Psychiatry 46:* 81–87, 1989.

heterogeneity, sample size, control group composition, and assay techniques; each of these concerns has been discussed in detail elsewhere.[34]

To control for most of these potential confounding factors and methodologic faults, a study was undertaken by our laboratory, which includes the largest sample of rigorously diagnosed patients with unipolar MDD (n = 91) and 91 age- and sex-matched controls.[47] All functional immune assays utilized dose-response curves, and hospitalized and ambulatory male and female patients with MDD representing a range of ages and illness severity were studied. No significant mean differences between the depressed patients and age- and sex-matched controls were found in the number of peripheral blood lymphocytes, T and B lymphocytes, and T4 (T helper) or T8 (T suppressor) cells. Figure 2 shows that mitogen-induced lymphocyte stimulation responses to PHA, ConA, and PWM for the depressed patients were similar to those of matched controls, with no significant mean differences between the groups. Natural killer cell activity also did not differ between the depressed patients and control subjects (Figure 3).

As an earlier study in our laboratory found decreased lymphocyte numbers and mitogen responses[37] in a sample of depressed patients who were older, predominantly male, severely depressed, and hospitalized, the latter factors may be related to immune system changes in depressed patients. Multiple regression analyses were conducted to investigate the contribution of age, sex,

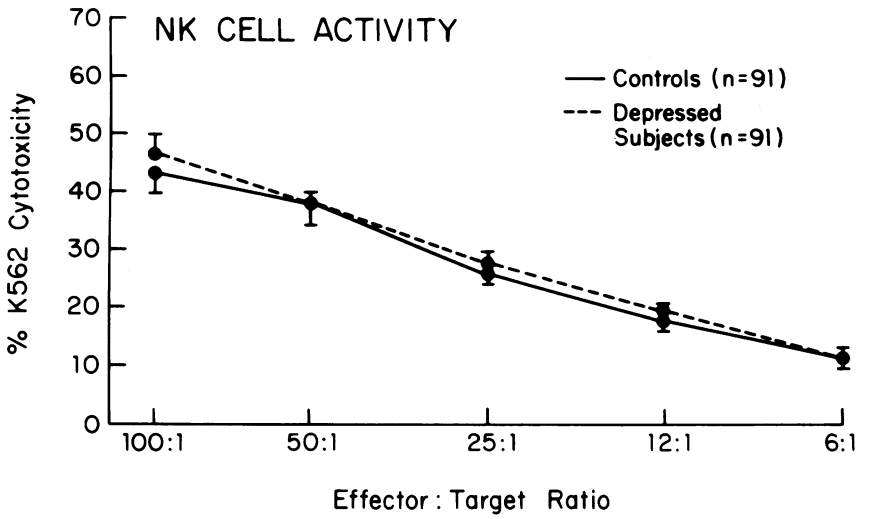

Fig. 3. Natural killer cell cytotoxicity in depressed patients and controls. Each point represents the mean ± SEM of percent specific cytotoxicity for each group. Reproduced by permission from Schiefer, S.J., Kelly, S.E., Bond, R.M., et al.: Major depressive disorder: role of age, sex, severity, and hospitalization. *Arch. Gen. Psychiatry 46:* 81–87, 1989.

severity, and hospitalization status to the immune measures of the depressed patients and the matched controls.[47] The analysis revealed significant age-related differences between the depressed patients and controls in the mitogen responses and the number of T4 lymphocytes. In contrast to age-related increases in mitogen responses in the controls, the depressed patients did not show increased lymphocyte responses with advancing age. Similar age-related differences were found between groups for T4 lymphocytes. The sample was not large enough, however, to determine if the age-related differences between depressives and controls were specific to any age group, e.g., the elderly or young adults and adolescents. In addition, severity of depression was significantly associated with the mitogen proliferative responses independent of age, and the more severe the depression, the lower the T-cell mitogen response.

These findings suggest that alterations in the immune system in MD/MDD do not appear to be a specific biologic correlate of major depressive disorder, but rather may occur in association with other variables that characterize depressed patients, such as age and symptom severity.

It is important to emphasize that the functional measures of the immune system used in the studies of depressive disorders only assess *in vitro* correlates to immune system activity. It has not been established, however, that the levels of mitogen-induced lymphocyte stimulation or natural killer cell activity are related to *in vivo* immune responses. Altered *in vitro* peripheral blood

lymphocyte responses may indicate that biologically important systemic events are occurring that may have a variety of consequences for the organism. Whether these will include changes in the ability to respond to infections or other *in vivo* challenges affecting health outcome remains to be determined. Future research concerned with brain, behavior, and the immune system requires more clinically relevant and specific immune measures.

Several biologic abnormalities have been observed in patients with MD/MDD that may be relevant to immune function. These abnormalities include alterations in the hypothalamic-pituitary-adrenal (HPA) axis[39] and disturbances in the function of the autonomic nervous system (ANS).[49] Immune cells have receptors for molecules derived from the HPA axis and the ANS, including corticotropin, B-endorphin, cortisol, and catecholamines.[50–52] *In vitro* or *in vivo* exposure of immune cells to these HPA- or ANS-derived substances are associated with alterations in immune function.[50,53] Furthermore, direct innervation of immune tissues by fibers emanating from the ANS has been described.[52]

Because corticosteroids, the end product of HPA activation, have potent immunoregulatory effects in humans that influence leukocyte traffic and function at both pharmacologic and physiologic concentrations,[54] the role of these agents in the relationship between depression and immune function has received considerable attention. Investigations of the relationship between HPA axis activity and immune measures in depressive disorders fall into two general categories depending on whether cortisol secretion was measured directly or whether the HPA axis response to dexamethasone was assessed. No differences in lymphocyte responses to the mitogens ConA, PHA, and PWM have been found between depressed patients with elevated or normal urinary-free cortisol levels.[55] In addition, no significant correlation between single-stick cortisol values and lymphocyte function has been demonstrated;[37,56,57] however, single-stick cortisol samples are not reliable indicators of overall cortisol secretion.[58]

At least three studies have compared immune measures in depressed patients with nonsuppression versus suppression on the dexamethasone suppression test (DST). Kronfol and colleagues[59] reported decreased lymphocyte percentages and absolute lymphocyte numbers in nonsuppressors, as well as a significant negative association between postcortisol and the total number of lymphocytes. In contrast, Murphy and associates[60] reported no differences in the number of lymphocytes in suppressors versus nonsuppressors, and Syvalahti and colleagues[61] observed no relationship between lymphocyte subset values or lymphocyte response to mitogens and dexametha-

sone suppression test status. It is important to note that the lymphocyte numbers utilized by Kronfol et al.[59] and Murphy et al.[60] were derived from retrospective chart reviews of white blood cell and differential counts, in contrast to Syvalahti et al.,[61] whose data were not retrospective and were based upon immunofluorescence techniques.

A review of the studies of neuroendocrine-immune interactions has not revealed a consistent or reproducible relationship between measures of the HPA axis and immune measures in patients with depressive disorders. A substantial amount of research concerned with the immune system in relation to depression has been conducted, and, by and large, the findings have been inconsistent and inconclusive. Immune changes in depressive disorders have not been as clear cut as early studies suggested.

Neuroendocrine-Immune Interactions

To understand more about neuroendocrine-immune interactions, our research has also investigated the effects of stress on the neuroendocrine and immune systems utilizing animal models. Secretion of corticosteroids has long been considered to be the mechanism of stress-induced modulation of immunity and related disease processes.[62,63] The regulation of immune function in response to stress, however, may not be limited to corticosteroids. In our laboratory, unpredictable, unavoidable electric tail shock has been shown in rats to suppress measures of the immune system, as determined by the number of circulating lymphocytes and PHA stimulation.[64] In an effort to determine if the adrenal is required for stress-induced suppression of lymphocyte function in the rat, we investigated the effect of stressors in adrenalectomized animals.[65] Four groups of rats were studied and consisted of nonoperated, adrenalectomized, sham adrenalectomized, and adrenalectomized animals with a corticosterone pellet. The four treatment groups, home-cage control, apparatus control, low-shock, and high-shock animals, were identical to those used in our previous study.[64] There was a progressive increase in corticosterone with increasing stress in both of the groups with adrenals; no corticosterone was detected in the adrenalectomized group, and the concentration of corticosterone in the adrenalectomized group that received the corticosterone pellets was constant. There was a significantly progressive stress-induced lymphopenia in both the nonoperated and sham-operated groups. There were no stress-related changes in lymphocyte number in either of the adrenalectomized groups. The findings of our study demonstrate that stress-induced lymphopenia in the rat occurs in association with stress-induced secretion of corticosteroids and can be prevented by adrenalectomy.

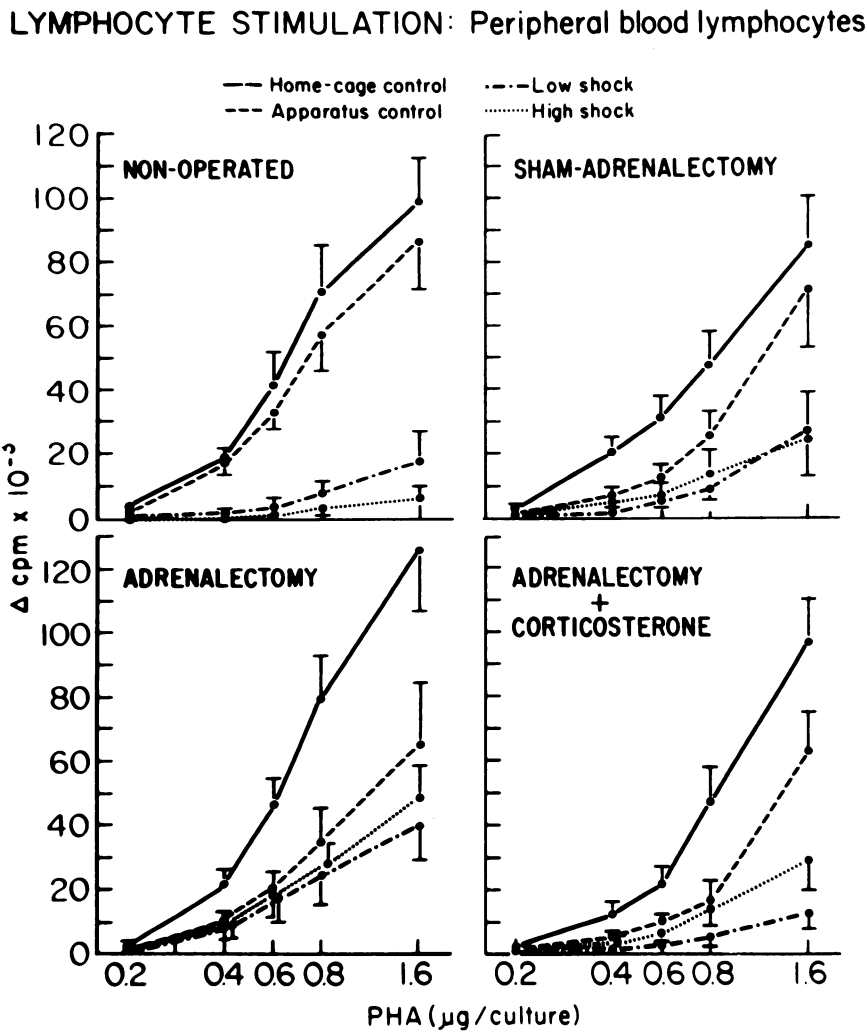

Fig. 4. Stimulation of isolated peripheral blood lymphocytes by PHA for each of the four operative groups and four treatment procedures. Data (means ± SEM) are represented as ΔCPM. Reproduced by permission from Keller, S.E., Schliefer, S.J., Liotta, A.S., et al.: Stress-induced suppression of immunity in adrenalectomized rats. *Science 221:* 1301–04, 1983.

The stressful conditions, however, suppressed the stimulation of lymphocytes by PHA in the adrenalectomized animals (Figure 4). The stressors similarly suppressed PHA responses in nonoperated animals, replicating our earlier report,[64] in sham-adrenalectomized rats and in adrenalectomized animals with steroid replacement. These findings demonstrate that stress-related adrenal secretion of corticosteroids and catecholamines is not required for the stress-induced suppression of lymphocyte stimulation by the T-cell

mitogen PHA in the rat. It may well be that there is an adrenal-independent stress-induced depletion of functional subpopulations of T cells or a selective redistribution of lymphoid tissues. A variety of other hormonal and neurosecretory systems may be involved in the adrenal-independent stress-induced modulation of T-cell function.

The findings of adrenal-dependent stress-induced lymphopenia and of adrenal-independent effects on lymphocyte stimulation indicate that stress-induced modulation of the immune system is a complex phenomenon involving several, if not multiple, mechanisms. Changes in thyroid hormones, growth hormones, and sex steroids have been associated with exposure to stressors, and all have been reported to modulate immune function.[66] Further, we,[17] as previously noted, and others[67] have shown that the hypothalamus, which plays a central role in neuroendocrine function, modulates both humoral and cell-mediated immunity. These findings suggest that a range of neuroendocrine processes may be involved in stress-induced alterations of the immune system.

Since a variety of hormones under pituitary control have been associated with immunoregulatory processes, our research program investigated the role of the pituitary in mediating stress-induced alterations of immunity. We studied the effects of a stressor on immune function in hypophysectomized rats.[68] Three groups of rats were studied, including nonoperated, sham-hypophysectomized, and hypophysectomized. The two treatments, home-cage controls and tail-shocked animals, were similar to those in previous studies. Plasma ACTH and corticosterone were increased in the stressed groups with pituitaries and were below detectable levels in the hypophysectomized animals.

In both the nonoperated and sham-hypophysectomized groups there was a stress-induced lymphopenia in the peripheral blood, as well as a stress-related decrease in the number of T lymphocytes and T-helper cells, but not in the number of T-suppressor cells. The number of B lymphocytes was not altered by the stressful condition. In the hypophysectomized animals no stress-related changes were found in the absolute number of lymphocytes or lymphocyte subsets. These findings demonstrate that the stress-induced lymphopenia in the rat is selective for T cells and specifically T-helper cells. Furthermore, the stress-induced lymphopenia is pituitary-dependent and is associated with increased levels of plasma ACTH and corticosterone, consistent with the observation that the number of circulating immunocompetent cells in response to a stressor is regulated by the hypothalamic-pituitary axis. The stress-related decrease in lymphocyte numbers from the peripheral blood may be

related to vascular margination or migration into the interstitial compartment, the lymphatics, or lymph nodes.

The stressful condition suppressed PHA-induced stimulation of peripheral blood lymphocytes in the hypophysectomized animals as well as in both control groups (Figure 5). These findings demonstrate that factors not of pituitary origin mediate the stress-induced suppression of peripheral blood lymphocyte proliferation. In addition to the hypothalamic-pituitary axis, the autonomic nervous system is another major stress-activated system, and stress-induced modulation of lymphocyte function may be related to neurotransmitter alterations. Utilizing a stressor similar to that employed in the hypophysectomy study, Weiss and Simson[69] found a marked depletion of

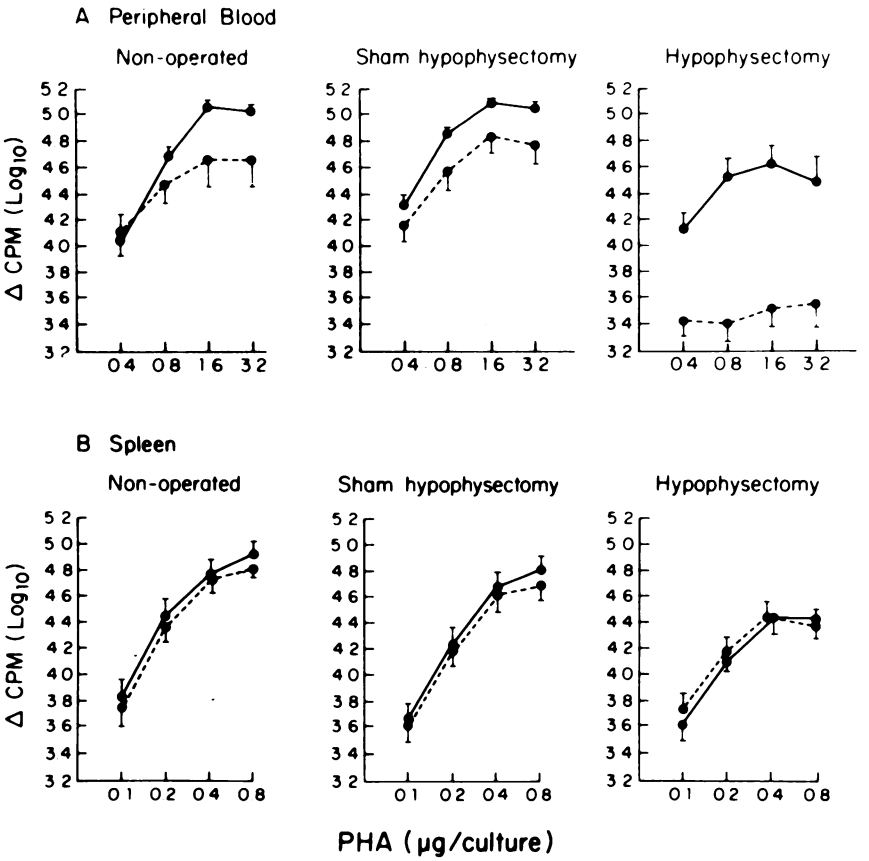

Fig. 5. Stimulation of peripheral blood lymphocytes (A) and spleen cells (B) by PHA for each of the three operative groups and two treatment procedures: Home cage controls—; shock – – –. Data (means ± SEM are represented as ΔCPM. Reproduced by permission from Keller, S.E. et al.: Stress-induced alterations of immunity in hypophysectomized rats. *Proc. Nat. Acad. Sci. 85:* 9297–9301, 1988.

norepinephrine in various regions of the rat brain, including the hypothalamus and locus ceruleus. It may well be that the findings of a pituitary-independent stress-induced suppression of peripheral blood lymphocyte proliferation is related to the involvement of central and peripheral catecholamine systems which have been shown to regulate immune processes.

Another interesting and major finding was that the magnitude of the stress-induced suppression of lymphocyte function in the hypophysectomized animals was significantly greater than in the control animals with pituitaries (Figure 5). These findings demonstrate that pituitary processes are involved in countering stress-induced immunosuppressive mechanisms. While the specific pituitary-dependent mitigating or compensating processes are not known, the findings suggest that a regulatory network of hormonal and nonhormonal systems is involved in the maintenance of immunologic capacity following exposure to stressors. The restraining influence of the pituitary on stress responses may be of relevance to the understanding of homeostatic maintenance of critical body functions.

It is of note that in all of our stress research with the rat,[64,65,68] including the hypophysectomy study (Figure 5), in contrast to the findings with peripheral blood lymphocytes, there were no systemic stress effects on splenic lymphocyte stimulation of PHA. The lack of a stress effect on the stimulation of splenic lymphocytes in contrast to peripheral blood lymphocytes may reflect differences in the various compartments of the immune system, each with its own microenvironment and subject to specific modulators and regulators.

Recently, our research has returned to consideration of the interactions between the neuroendocrine system and the immune system as a means of further understanding the relationship between adrenal steroid hormones and lymphocyte function. Two separate high affinity receptors for glucocorticoids have been characterized,[70] and both type I (also referred to as mineralocorticoid receptors) and type II (also referred to as glucocorticoid receptors) adrenal steroid receptors have been demonstrated in human peripheral blood lymphocytes.[71,72] Few studies have evaluated the relative presence of type I and type II receptor binding in other immune compartments. Miller and colleagues[73] in our laboratory have found that type I and type II receptors are differentially expressed in the spleen and thymus. Both type I and type II receptor binding has been found in the spleen, whereas only type II binding was detected in the thymus. This differential expression of receptor may confer on these tissues different sensitivity or responsivity to glucocorticoids

and thereby explain why one immune compartment may respond differently following stress compared to another.

Miller and associates[73] have also noted that the occupation and activation of the receptors in immune tissues differs from adrenal steroid receptors in the hippocampus. Following one hour of restraint stress, both type I and II receptors in the hippocampus were significantly occupied and activated. Despite peak levels of corticosterone, glucocorticoid receptors in the spleen, thymus, and pituitary showed no evidence of activation following stress. These findings demonstrate that there is not only differential expression but that there is also a considerable degree of heterogeneity in the activation of receptor subtypes in immune, pituitary, and hippocampal tissue following stress. Further studies are being conducted to determine the factors which may contribute to differences in receptor binding as well as the relation of receptor activation to immune function. Future directions for research concerned with brain, behavior, and the immune system should include the pursuit of more basic knowledge about brain-neuroendocrine-immune interactions. The availability of such basic information may lend itself to further understanding of the relationship among clinical behavioral conditions, the immune system, and health and illness.

A lack of conceptual clarity has complicated the interpretation of findings in many of the studies concerned with brain, behavior, and the immune system. This is in part due to the fact that the association among brain, behavior, and the immune system may be viewed from at least three conceptual frameworks. These conceptualizations have been discussed in detail elsewhere[34] and include: the role of immune function in health and illness, neuroimmunology, which primarily concerns itself with disordered immune function that affects the nervous system and alters central nervous system activity, e.g., autoimmune and viral processes and neural-immune interactions. In view of the conceptual complexity involved in the investigation of brain, behavior, and the immune system, future directions for research in this area should include studies that incorporate sound research conceptualizations and the appropriate experimental design and methods to answer the questions being asked. Future research concerned with brain, behavior, and the immune system offers an exciting opportunity to increase our knowledge in neurobiology and immunology and to provide a foundation for clinically relevant studies.

ACKNOWLEDGEMENTS

I thank Susan S. Kang for her assistance in preparing this manuscript and Dr. Andrew H. Miller for his helpful comments.

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