Psychoneuroimmunology of Depression

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INTRODUCTION

The central nervous system is hypothesized to have a role in the modulation of immune function. This chapter provides an overview of the association between psychological factors and immunity, concentrating on the immunological alterations of depression. In addition, experimental evidence is reviewed of the neural and endocrine mechanisms that have been proposed to alter immune function in depression. Finally, because psychological distress has recently been associated with an increased susceptibility to infectious disease and possibly risk of cancer, the possible health implications of depression or stress-associated immune changes are discussed. Before describing the interactions between the brain, behavior, and immunity, a brief overview of the immune system is provided (see Interactions Between the Nervous System and the Immune System: Implications for Psychopharmacology).

THE IMMUNE SYSTEM

The immune system functions to discriminate "self" from "nonself" cells, protecting the organism from invasion by pathogens, such as viruses and bacteria, or from abnormal internal cells such as cancer cells. These functions are closely regulated and performed without damage to the host, although an overresponsive immune system is purported to lead to autoimmune disease in which the organism's own tissues are attacked.

The organs of the mammalian immune system include the thymus, spleen, and lymph nodes. The working cells of the immune system are represented by three distinct populations: T cells, B cells, and natural killer (NK) cells. Immune responses are typically divided into two important components: cellular immunity and humoral responses. There is evidence that T cells and B cells interact and cooperate in many cellular immune responses and in most humoral immune responses, although cellular immunity is thought to be mediated primarily by T lymphocytes and humoral responses by B lymphocytes and their soluble products.

The T lymphocytes develop from stem cells in the bone marrow and migrate to the thymus, where they mature into several subsets including the cytotoxic T cell, T helper cell, and T suppressor cell. These T cells circulate into the periphery and are found in the lymph nodes, blood vessels and spleen. Briefly, the cytotoxic T cell is characterized by its ability to seek out and destroy either cells infected with viruses or tumor cells that have acquired foreign nonself antigens. In the development of the cytotoxic T cell response, a foreign antigen is first encountered and incorporated onto the surface of an antigen-presenting cell such as a macrophage. After the antigen is presented to the T cell, is recognized, and is bound by a specific receptor on the T cell, then the T cell multiplies and becomes capable of attacking any cell that presents that specific foreign surface antigen. Other types of T lymphocytes, such as the T helper

cells, secrete interleukin-2 (IL-2) and regulate the proliferative response of the T cell to antigenic stimulation. Reexposure of the cytotoxic T cell to an antigen produces a more rapid and extensive reaction than that found upon initial presentation.

Interacting with the T helper and T suppressor cell, the B cell is primarily involved in the humoral response. Like the T cell, the B cell arises from a precursor stem cell in the bone marrow, although in humans its site of maturation remains unknown. Following initial antigen processing by the macrophage, the antigen–major histocompatibility complex (MHC) on the accessory cell surface engages the receptors of an appropriate T helper cell antigen and the macrophage releases interleukin-1 (IL-1). Thus stimulated the T helper cell proliferates by forming a clone and secretes factors, such as IL-2, that stimulate T-cell and B-cell growth. The activated B cell proliferates and differentiates to antibody secreting status, switching with the help of interleukin-4 (IL-4) from immunoglobulin M (IgM) secretion to synthesis of other classes of antibodies such as immunoglobulin G (IgG).

In addition to the T and B cells, a distinct subpopulation of lymphocytes comprised of NK cells has been described. The NK cell is immunologically nonspecific and does not require sensitization to specific antigens to perform its cytotoxic activity. Thus, the NK cell responds to a variety of cell surface markers, as long as the markers differ from "self" markers, and lyses a wide variety of cell types. Although the role of the NK cell in tumor surveillance remains controversial, substantial evidence has demonstrated the importance of the NK cell in the control of herpes and cytomegalovirus infections in man and animals.

Regulation of immune responses involves the secretion of cellular factors or lymphokines. These lymphokines together form a network of regulatory signals that show considerable overlap in activity and patterns of synergism as well as antagonism. For example, the lymphokine IL-1 is produced by nearly all immunological cell types including NK cells, T and B lymphocytes, brain astrocytes, microglia, and macrophages. Interleukin-1 acts mainly as an endogenous adjuvant serving as a cofactor during lymphocyte activation, inducing the synthesis of other lymphokines and the activation of resting T cells. For example, IL-1 stimulates T lymphocytes to synthesize and release IL-2, and IL-1 further acts on NK cells to induce the expression of the IL-2 receptor. Binding of IL-2 by its receptor on the NK cell is a crucial step in activating such cytotoxic cells (predominantly large granular lymphocytes) to form lymphokine-activated killers that are able to lyse a wide range of target cells in a non-MHC-restricted manner.

MEASURES OF IMMUNE FUNCTION

The immune system can be evaluated by measures that assess the *number* of different cell types as well as the *function* of various components of cellular and humoral immunity. To quantitate the number of cells in various subpopulations, specific monoclonal antibodies are available that bind to unique surface markers on cell types such as T helper, T suppressor, and NK cells. Whereas enumeration of cell types reveals the balance of different cell types needed for the optimal immune response, numbers of different cell types do not necessarily correlate with functional capacity, and changes of cell numbers in the peripheral circulation may merely reflect a redistribution of cell types from various immune compartments (60).

Measurement of the *function* of the immune system can involve in vivo and in vitro techniques. One in vivo assay of immunity includes measurement of the delayed-type hypersensitivity (DTH)

response following administration of skin tests. This in vivo technique provides valuable data about the physiological response of the organism to an antigenic challenge and has been proposed to be more relevant in the clinical assessment of immunocompetence than in vitro measures of immune function (32). For the assessment of the DTH response, a series of antigens (i.e., tetanus, diphtheria, streptococcus, tuberculin, candida, trichophyton, proteus) and a glycerin control are applied in a standardized fashion to the forearm. The induration response at each site of antigen exposure is measured typically after 48 hr and the number of antigenic responses and size of induration at each site is recorded. Cutaneous anergy is indicated by the absence of an induration response to any of the seven test antigens.

Immunization with a novel antigen and evaluation of antibody response is another technique that has been used to assess immune function in vivo. For example, Glaser et al. (27) have used the hepatitis B recombinant vaccine and quantitated rates of seroconversion by assaying serum antibody titers to the hepatitis B surface antigen. In addition to the use of well-recognized vaccines such as hepatitis B or pneumovax, the use of a novel protein antigen (keyhole limpet hemocyanin) has also been proposed to evaluate in vivo the kinetics and magnitude of this biologically important response (33).

An indirect in vivo assessment of cellular immune function includes the measurement of antibody titers to latent viruses. The cellular immune response is thought to be important in controlling latent viral infections, and reactivation of viruses, such as herpes viruses, can occur during conditions in which cellular immunity is compromised. In turn, synthesis of virus or viral proteins is increased and elevations in antibody titers are detected in the serum.

Two in vitro correlates of cellular immune function, mitogen-induced lymphocyte proliferation and NK cell activity, have been widely used to assess the cell-mediated immune system of depressed patients. Both of these assays evaluate the function of cells ex vivo outside the body. Mitogen-induced lymphocyte stimulation determines the proliferative capacity of lymphocytes following activation in vitro either with plant lectins such as concanavalin A (Con A) or phytohemagglutinin (PHA) which predominantly activate the T lymphocyte to divide or with pokeweed mitogen that induces proliferation of B cells. The proliferative response is quantitated by the cellular incorporation of radioactively labeled thymidine or idoxuridine into newly synthesized deoxyribonucleic acid (DNA). The other frequently used in vitro assessment of cellular immunity involves measurement of NK activity. Assay of NK lytic activity is carried out by the coincubation of isolated effector lymphocytes with radioactively labeled target tumor cells. The release of radioactivity by the lysed target cells is proportionate to the activity of the effector NK cells.

Assay of levels of lymphokines (IL-1, IL-2, and interferon) in the plasma or following lymphocyte stimulation has been employed recently to quantitate possible alterations in the regulation of these cellular factors, which are important in humoral and cellular immune responses (26). Interleukin-1 promotes lymphocyte differentiation via increased expression of IL-2 receptors, whereas IL-2 provides a signal for the proliferation and differentiation of immune cells. Assay of these lymphokines is proposed to provide information about the immunological mechanisms that may be related to changes in ex vivo measures such as lymphocyte proliferation or NK activity (26, 55).

STRESS AND IMMUNITY: ANIMAL MODELS OF DEPRESSION

Effects of Stress on Cellular Immune Responses

Behavioral responsiveness to inescapable aversive stimulation has provided an animal model to investigate clinical depression. Administration of aversive stressors, including sound, rotation, intermittent shock, and forced immobilization, have been found to alter in vitro correlates of cellular immunity (i.e., lymphocyte responses to mitogen stimulation and/or NK activity) in a manner that depends on dose- and time-response profiles. For example, using either an audiogenic stressor or intermittent footshock repeated at daily intervals, several studies showed that acute immune suppression occurred after only one or two exposures to the stressor and was followed by an increase or enhancement of lymphocyte proliferation or natural cytotoxicity if the stressor was repeated or prolonged (41, 42). The dose of the stressor is also associated with the degree of immune change; a progressive decrease of PHA-induced proliferation is found in animals that receive an increasing severity of stress from low- and high-level electric tail shock (46).

Cellular in vivo immune responses show a similar reduction of function following the administration of aversive stressors. Delayed hypersensitivity reactions are decreased in mice exposed to heat stress, and the graft-versus-host response is suppressed in animals subjected to limited feeding, an effect that is independent of adrenocortical levels (see also Neuroendocrine Interactions, Interactions Between the Nervous System and the Immune System: Implications for Psychopharmacology, and Stress, for related discussions).

Stress and Humoral Immune Responses

Studies of the effects of stress on the immune system have recently suggested the importance of measuring immune function in vivo, rather than by assay of in vitro parameters of cellular immunity (56). For example, in vivo assessment of an antibody response to a novel antigen reveals the integrated action of the intact immune system, which can not be fully evaluated when multiple parameters of cellular immune function are separately assayed in vitro (56). In addition, measurement of an in vivo response is carried out within the internal milieu, providing a model that can test the physiological role of neurotransmitters and neuroendocrine hormones in the modulation of immune function during stress.

Administration of aversive stressors such as tail shock, footshock, or social status change has been found to produce a reliable suppression of a specific IgG antibody response following immunization with the T-cell–dependent antigen keyhole limpet hemocyanin (KLH). Likewise, following intraperitoneal administration of sheep red blood cells (SRBC), the plaque-forming cell response was reduced in monkeys exposed to aversive stimuli, in mice housed in high- versus low-density group-housing, and in mice subjected to changes in housing condition (either from individual to group housing or from a group to an individual cage).

A critical period exists during which the humoral response is vulnerable to the effects of stress, although disparate results have been reported. Restraint produced a significant reduction in antibody response to SRBC only if the stress is applied before, but not after, immunization; rotational stress depressed antibody-forming cell responses to SRBC when administered 24 hr after immunization but not at the time of immunization, whereas a critical period extending up to 72 hr following immunization was described for footshock. Because inescapable shock has been found to alter mesenteric lymph node lymphocyte subpopulations as well as specific antibody

responses in a time-dependent manner (changes occurring immediately following stress become absent 48 hr after stress), the temporal profile of stress-induced immune suppression could be due to the differential effects of stressor on the alteration of lymphocyte subpopulations as reflected by an increased T helper/T suppressor/cytotoxic lymphocyte ratio.

The effects of stress on antibody response is also antigen-dose dependent. In other words, the effects of stress such as footshock are found only when the amount of antigen used is close to a physiological concentration not when suprathreshold amounts of antigen are used to evoke an exaggerated, rapid response (33).

Stress Alterations of Accessory Cell Function

Accessory cells, such as macrophages, which are essential for the modulation of immune responses including lymphocyte proliferation and antibody responses by release of IL-1 and prostaglandins, are likely to play a role in stress-induced alterations of immune responses. During an antibody response, the macrophage expresses class II MHC gene products, processes and presents antigen, and facilitates activation of T helper cells. To evaluate whether macrophage function might be a target of stress and lead to suppression of antigen-specific T-helper-cell function, we have used irradiated spleen cells from either amphetamine-stressed or control animals to present antigen to antigen-specific T-cell hybridomas. Interleukin-2 production by T-cell hybridomas was abolished when antigen-presenting cells from amphetamine-stressed animals were used, suggesting that stress-induced defect in antigen-specific proliferation and/or IL-2 production may be at the level of antigen presentation by the macrophage. Other recent data have further demonstrated that stress produces a dysregulation of macrophage function as measured by enhanced IL-1 and prostaglandin E_2 production and diminished interferon-induced class II MHC gene product expression. Diminished class II MHC expression is thought to compromise antigen presentation and T-helper-cell activation.

Stress Alterations in Immune Cell Second-messenger Systems

Although data have supported the hypothesis that macrophage function may be impaired by stress, the possibility of some additional defect in T-cell function cannot be excluded. Activation of lymphocytes as measured by lymphocyte proliferation is a multistep process, and thus stress-induced suppression of this immune activity might also be mediated by alterations of discrete intracellular biochemical events that occur immediately after mitogen binding. For example, mitogen binding to the T-cell receptor is transducted via a bifurcating pathway from the hydrolysis of phosphatidyl inositol biphosphate (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 in turn elevates free calcium while DAG activates protein kinase C (PKC). Finally, activation of calcium-dependent proteins increases synthesis of the IL-2 receptor and the lymphokine IL-2 (26).

Because of the important role of the early activating biochemical steps in the proliferative response of lymphocytes to mitogens, initial studies have begun to explore whether the effects of stress on lymphocyte function are mediated by alterations in calcium mobilization or calcium-dependent biochemical mechanisms. Indeed, both acute and chronic restraint stress have been found to suppress mitogen-stimulated increases in calcium, suggesting that stress induces an inhibition of calcium mobilization in lymphocytes and attenuates this obligatory signal for certain signal-transduction pathways. However, other data suggest that stress-induced defects in T-cell

proliferation are not solely due to alterations in free-calcium mobilization but rather may be due to intracellular biochemical changes in addition or subsequent to early events driven by calcium release. For example, diminished lymphocyte responses following stimulation with either calcium ionophores (i.e., ionomycin or A23187 that releases free intracellular calcium) or tetradecanoylphorbol acetate (TPA) which activates PKC are found in stressed animals. Together, these data suggest that stress may induce some defect in surface-receptor signal transduction, but that an additional intracellular defect is present at a site beyond the action of calcium or PKC, possibly in the synthesis of IL-2 and IL-2 receptors. Interestingly, a stress-induced suppression of IL-2 production with PHA has not been demonstrated, and the addition of IL-2 failed to correct stress-related suppression of blastogenesis.

Psychological Predictors of Stress-induced Immune Alterations

Stressor Predictability and Controllability

Stressor characteristics, such as severity and type of stressor and/or time response, partly determine alterations of a wide range of cellular and/or humoral immune parameters. However, the psychological state of the animal in response to the stressor has also been related to the immunological consequences of the stress. Maier and Laudenslager (56) found that rats exposed to inescapable, uncontrollable electric tail shock have reduced lymphocyte activity, whereas animals that received the same total amount of shock but were able to terminate it did not show altered immunity. Furthermore, stress predictability, such as a warning stimulus preceding inescapable footshock, completely reverses the shock-induced suppression of lymphocytes. Finally, consistent with the hypothesis that fear ultimately determines the amount of immunosuppression induced by aversive stimuli, a nonaversive stimulus that is associated with electric shock can be conditioned to impair lymphocyte proliferation independent of any physical effects of aversive stimulation.

Role of Early Life Experiences

Premature maternal separation produces alterations in a number of measures of immune function, such as lymphocyte proliferation, macrophage responses, decreased levels of complement proteins and immunoglobulins, an inhibited capacity to mount antibody responses to the bacteriophage {ewc MVIMG, MVIMAGE,!times.bmp}174, increased hemolytic complement activity, and greater delayed hypersensitivity responses to dinitrochlorobenzene (8). In addition to the acute effects of maternal separation on immune function in the infant animal, these immune alterations persist into adulthood, leading to changes of the immune system and possibly of host resistance to infectious disease in the adult animal (52).

Role of Affiliative Social Behavior

The impact of psychosocial stress on immune function may also be buffered by social environment and/or the social behavior of the animal at the time of exposure to the stressor. Administration of a chronic (2 years) social stressor involving housing either in a stable or in a changing social group produces a decrement of cellular immune responses in monkeys that is consistent with the effects of other social stressors. Of unique interest, those animals who showed affiliative behaviors while undergoing social stress had greater values of lymphocyte proliferation as compared to less affiliative animals. In other words, social behaviors that are affiliative may serve to protect animals from the immunosuppressive effects of chronic social stress.

STRESS AND IMMUNITY: CLINICAL FINDINGS RELEVANT TO DEPRESSION

Life stressors such as bereavement, marital separation, academic examinations, or the stress of Alzheimer spousal caregiving have all been shown to have effects on immune function. Individuals undergoing bereavement show alterations of cellular immunity including suppression of lymphocyte responses to mitogenic stimulation, reduction of NK cell activity, and alterations of T-cell subpopulations. Consistent with the observation that severity of depressive symptoms is a reliable negative correlate of altered immunity in depressed patients (as described below), psychological response to bereavement, not merely the event, appears to contribute to the immune changes in severe life stress. Likewise, using various measures of humoral and cell-mediated immunity, reduced lymphocyte responses to the mitogen PHA were found in bereaved subjects who had high depression scores but not in those bereaved subjects who had few signs of depression as compared to controls. Similar to the effects of conjugal bereavement, marital disruption in women and in men is associated with a suppression of proliferative lymphocyte responses to Con A and PHA and with increased antibody titers to Epstein-Barr virus (EBV). These immune alterations in separated and divorced individuals are correlated with depressive symptoms and measures of psychological attachment.

Psychological stress during relatively minor aversive events such as academic examinations is also temporally associated with higher white blood cell counts, an increased absolute lymphocyte count, a reduction of lymphocyte proliferation and NK activity, and alterations in the regulation of IL-2 and IL-2 receptor on peripheral blood lymphocytes (26). Furthermore, these alterations of cellular immunity in medical students undergoing examination stress have been hypothesized to be of health significance since antibody titers to EBV, cytomegalovirus (CMV), and herpes simplex virus (HSV) are significantly increased during the first day of examination as compared to either the month before examination or upon return from summer vacation. Finally, Glaser et al. (27) has further demonstrated that medical students who are less stressed and anxious are more likely to show a shorter time to seroconversion following vaccination with the hepatitis B vaccine.

Chronic stressors that last over periods of one or more years not only increase the risk for depression but also are likely to result in alterations of immune function. As compared to age-matched controls, family caregivers of Alzheimer patients are more likely to be psychologically distressed and to have significantly higher antibody titers to EBV and reduced percentages of total T lymphocytes and T helper cells. Additional observations have found higher rates of syndromal depressive disorders and impairment of cellular immunity (i.e., increased EBV titers and poorer lymphocyte proliferation to PHA and Con A) in spousal caregivers (47). An important clinical implication of these findings has suggested that these immunological alterations are longitudinally associated with more days of infectious illness, primarily upper respiratory tract infections (47).

DEPRESSION AND IMMUNE CHANGES

Summary of Findings

Studies of immunity in depression have been concerned primarily with the evaluation of enumerative (numbers of circulating white blood cells or lymphocyte subsets) and/or in vitro correlates of cellular immune function (lymphocyte responses to mitogens and NK activity).

Because of the disparate results from several dozen studies conducted since 1978, three comprehensive reviews have recently been published (31, 71, 76), although the conclusions reached from these reviews using overlapping studies are not in agreement. For example, Stein et al. (71) used a tabular summary of the findings from 22 studies and concluded that "the findings have been inconsistent and inconclusive." Enumerative measures do not distinguish depressed patients from controls and ". . . no consistent or reproducible alterations of functional measures of lymphocytes" have been reported. In contrast, Weisse (76) evaluated 18 studies using similar vote-counting methods and concluded that "indexes of immunocompetence [as reflected in lymphocyte responses to mitogen proliferation] are lower among the clinically depressed." Finally, Herbert and Cohen (31) evaluated essentially the same series of studies (of the 36 studies reviewed by Herbert and Cohen, 18 overlapped with Weisse and 22 with those reviewed by Stein et al.) and concluded using a metaanalytical approach that "clinical depression is reliably associated with decreases in all of the measures of lymphocyte function" including NK activity and responses to PHA, Con A, and pokeweed mitogen. Furthermore, in an analysis of 15 methodologically restricted studies that assessed depression using diagnostic criteria, included a comparison control group, and fulfilled several other criteria of methodological rigor (i.e., age and gender of depressed and control subjects indicated, age and gender matching of depressed patient– controls or statistical control for age and gender, drug-free assessment of depressed patients, and psychological and physical health of controls), effects sizes increased and were "particularly robust" ranging from -0.24 to -0.45 (31).

The following summary further reviews alterations of cellular immune function in depression but is restricted to those studies published since 1978 that have employed several methodological strengths (31) and used interassay controls to evaluate and/or control the effects of data variance in the measurement of mitogen responses or NK activity (71). Briefly, because of the essential importance of diagnostic homogeneity as well as control for confounding influences of medication, age, and gender in the interpretation of immune alterations in depression, the present review includes those studies that have evaluated samples of depressed patients who fulfilled diagnostic criteria (either DSM-III or Research Diagnostic Criteria) for major depression, were assessed while psychotropic medication free for at least one week, and were compared with healthy age- and gender-matched comparison controls. In addition, because of the recognized variability of functional immune assays and the need for interassay controls (71), this summary only includes those studies that have been conducted using a matched subject–control pair design and/or laboratory controls with one or more cryopreserved standards. Of the 38 studies evaluating lymphocyte proliferation or NK activity in depression, 12 studies (16, 17, 18, 34, 35, 38, 40, 50, 62, 65, 66, 67) fulfill these methodological criteria. With the addition of Irwin et al. (34) and Darko et al. (17), the 38 studies reviewed herein overlap with those 36 publications reviewed by Herbert and Cohen (31); 22 overlap with those reviewed by Stein et al. (71); and 18 with Weisse et al. (76).

Mitogen-induced lymphocyte proliferation was assessed in five (16, 17, 65, 66, 67) of the 12 depression-immune studies fulfilling the specified stringent methodological criteria. Of these five studies, one study has found a reduction of PHA-, Con-A-, and pokeweed mitogen-induced lymphocyte proliferative responses in depressed patients (67). The other four studies found no group difference in this immune response between depressed patients and controls, although Schleifer et al. (65) found that severity of depression was associated with suppression of proliferative responses within the depressed sample.

For the measurement of NK activity, eight studies published since 1978 (18, 34, 35, 38, 40, 50, 61, 65) have employed interassay controls in addition to fulfilling the other important methodological criteria of diagnostic homogeneity, medication-free status, and age and gender matching with control comparison subjects. Of these eight studies, seven have reported a reduction of NK activity in depressed patients as compared to controls (18, 34, 35, 38, 40, 50, 61). Although the one study by Schleifer et al. (65) failed to find a reduction of NK activity in the depressives, a modest trend was evident between the severity of depressive symptoms and NK activity (Keller, *personal communication*).

Methodological Issues

Several factors such as sample differences in age, gender, hospitalization stress, concurrent life stress in either the depressed patients or controls, comorbidity in the depressives, or severity of depressive symptoms are likely to increase the immune variability in depressives and contribute to different results even in series that are restricted to those carefully controlled studies. For example, immune decrements occur in aged individuals (34), and depressed patients who are older may be more vulnerable to the effects of depression (33). Schleifer et al. (65) have shown that depressed people show decreased proliferative responses to mitogens with advancing age, whereas controls appeared to show increases. Furthermore, metaanalytical techniques have demonstrated that the effect size of depression-related reduction of immune function is reliably stronger in older clinically depressed people than in young subjects (31). However, in the 12 studies reviewed here, subjects were on the average middle-aged, and cellular immune measures often do not show either increased variability or declines in persons who are younger than aged 65 years (34).

Gender appears to have a striking influence on depression-related reduction of NK activity. Many studies have focused only on men and typically found a reduction of NK activity (34, 35, 38, 40) whereas the largest study that was conducted using predominantly female subjects found no difference in cellular immunity (65). Evans et al. (18) have separately compared gender differences for NK activity and NK cell counts and reported that depressed men exhibit significant reduction in these immune measures. Female depressed patients have similar levels of NK activity as well as NK numbers compared to female controls, suggesting the possibility that major depression has differential effects on NK cell function in male and female subjects. Indeed, *increased* NK activity was reported in female depressives which was speculated to be due to direct effects of progesterone, or possibly progesterone-mediated antagonism of glucocorticoid suppression on cellular immune function (57).

Hospitalization stress has been proposed to exacerbate the relationship between depression and immunity, although this relationship may be due to increased severity of depressive symptoms in hospitalized depressives (66). However, hospitalization status was not found to statistically contribute to mitogen-induced lymphocyte proliferation or NK activity (65), and schizophrenic patients undergoing the stress of hospitalization failed to show a reduction of cellular immune function even though a group of similarly hospitalized depressed inpatients did show such a reduction of immunity. Together, these data suggest that the effects of depression on immunity are probably not from nonspecific effects of hospitalization.

Considerable evidence has found that life stress can alter immune function. Depressed patients often undergo increased numbers of life events secondary to their affective illness and

depression-related reduction of cellular immunity could possibly be a result of the effects of life stress, not depression (35). Conversely, approximately 10% of control subjects who volunteer for clinical research and are free of any psychiatric diagnosis are found to be undergoing severe life stress as identified by structured life event interviews (38). Failure to assess life stress in depressed patients and controls could lead either to the inclusion of depressed patients who show a reduction of immunity secondary to life stress or alternatively to the enrollment of stressed controls who show stress-related reduction of immune function. To evaluate the relative contribution of stress to depression-related immune decreases as well as to strengthen the methodological rationale for assessing life stress by structured interviews in controls, one study of immune function in depression has rated severity of life stress in depressed patients and controls and demonstrated that both depression and life stress have independent, but not interactive effects on NK activity (38). In other words, depressed patients who were not severely stressed had a similar reduction of NK activity as that found in depressed patients who were also stressed. Psychiatric controls who are undergoing stress also show reduced immunity similar to depressed patients even though these controls reported no significant depressive symptoms. Important for the design of further clinical studies, inclusion of such normal controls in the comparison group could potentially obscure immunological differences between the depressed and control groups.

Increased use of tobacco and/or alcohol might worsen depression-related immune changes; depressed patients increase their tobacco smoking and over 30% escalate their alcohol drinking during a depressive episode. Despite the potential importance of alcohol and tobacco smoking to immune alterations in depression, evaluation of the contribution of either substance to altered immunity during depression is essentially limited to a few studies. In one study (38), smoking was found to be a strong predictor of the increased number of circulating neutrophils and lymphocytes in the depressives, but cigarette use did not affect the association between depression and NK activity. Indeed, others have found that depressed patients who are smokers have higher levels of lytic activity than tobacco smoking controls (23).

The effects of current alcohol intake and past long-term alcohol abuse on immune function in depressed patients has also been evaluated (35, 38). Although alcohol consumption in the 6 months prior to immune assessment was a significant predictor of total leukocyte and neutrophil cell counts similar to the effects of smoking, histories of recent alcohol use were not associated with NK activity (35). Past heavy alcohol consumption as reflected by histories of alcohol abuse and alcohol dependence however, did produce a graded decrement of NK activity beyond the effects of depression alone (35). Nevertheless, depression had an effect on NK activity independent of the effect of past alcoholism.

In addition to careful assessment of substance comorbidity, psychoimmunological studies of psychiatric patients with mood disorders need to evaluate comorbidity for other psychiatric disorders and the potential influence of this comorbidity on immune function. For example, Andreoli et al. (1) investigated the contribution of the additional presence of panic disorder to the variability of lymphocyte proliferation in depressed patients and found that depression with simultaneous panic disorder had a greater number of T cells and PHA mitogen responses than depressed patients without comorbid panic disorder.

Severity of depressive symptoms is a correlate of immunity and may be a more important determinant of altered immunity than depression diagnostic classification per se. For example in

Schleifer et al. (65) depressed patients had similar levels of lymphocyte proliferation compared to controls, yet severity of depressive symptoms was associated with suppression of mitogen proliferation. Furthermore, Schleifer et al. (66) suggested that ambulatory depressives have similar levels of immune function compared to controls due to their modest symptom severity. In our studies of NK activity in depression, severity of depressive symptoms has been reliably associated with a reduction of NK activity (35, 38, 65), a finding that has been replicated by Evans et al. (18) and strengthened by the metaanalysis reported by Herbert and Cohen (31). Finally, Irwin and colleagues used a longitudinal case control design and found that NK activity increases when symptom severity resolves following clinical psychopharmacological treatment (37).

To extend these findings regarding symptom severity and to evaluate the effects of diagnostic subtype on immune function, Hickie et al. (32) have assessed delayed-type hypersensitivity (DTH) response and PHA-induced lymphocyte proliferation in 57 patients with major depression as compared to age and gender matched controls. Patients with melancholic depression had a higher rate of abnormally reduced DTH induration diameter when compared with both nonmelancholic patients and with age- and gender-matched controls; this difference could not be accounted for by age, severity of depressive symptoms, hospitalization status, or weight loss. A similar pattern of results was found for in vitro assessment of immunity by PHA lymphocyte proliferation in that melancholic depressed patients showed impaired blastogenic response when compared with those with nonmelancholic depressive disorder. The significance of diagnosis of melancholia in determining impaired cellular immunity as measured by mitogen responsiveness is supported by two previous studies (54).

Finally, in an effort to broaden immunological assessment and to explore whether mediators important in the regulation of cellular immune responses are altered in melancholic depression, production of IL-1β and soluble IL-2 receptors in PHA culture supernatants has been determined in depressed patients with melancholic subtype (55). Depressed melancholic patients showed higher accumulation of IL-1β and possibly IL-2 receptors. Furthermore, dexamethasone suppressed IL-1β and IL-2 receptor production in controls but failed to alter responses in depressives. These findings, together with evidence of an increased number of pan T, pan B, and T-suppressor/cytotoxic cells in melancholics, have suggested to Maes et al. (55) that melancholics exhibit a systemic immune activation. Contrary to the hypotheses that blunted lymphocyte responses or NK activity are mediated through a deficit in T helper cells and/or lymphokine stimulation (65) or by inhibiting effects of various neuroendocrine signals (34), Maes et al. hypothesized that lower ex vivo mitogen responses may be due to immune activation in vivo, which leads to increased in vivo production of soluble IL-2 receptors inducing a state of relative deficiency of IL-2, which is necessary for proliferation.

MECHANISMS OF DEPRESSION-RELATED IMMUNE ALTERATIONS

Behavioral Factors

Sleep

A link between sleep and immune-related activity has been demonstrated by the ability of immune mediators to alter sleep activity in animals. For example, several cytokines such as IL-1, interferon-α_2, and tumor necrosis factor have the capacity to enhance slow-wave sleep, and a

growing body of evidence suggests that IL-1 is directly involved in sleep regulation in humans and animals (51). Antibodies to IL-1 reduce sleep in normal rabbits and the specific IL-1 receptor antagonist blocks the somnogenic effects of IL-1, inhibiting IL-1–induced increases of non-REM sleep without reversing IL-1 induced suppression of REM sleep. Additional data from Krueger (51), and colleagues have suggested that IL-1 may have a physiological role in sleep regulation since the IL-1 receptor antagonist transiently reduces non-REM sleep in rabbits. Studies have further demonstrated a wide distribution of IL-1 mRNA in the brain (19), the presence of IL-1 immunoreactivity in neurons of the human hypothalamus, and the secretion of IL-1 into the CSF and plasma during sleep–wake cycles (58).

In addition to the likely direct effects of IL-1 on sleep regulation, IL-1 may be involved in the regulation of other substances that either enhance or inhibit normal sleep. For example, IL-1 enhances the release of corticotropin-releasing factor (CRF) and CRF, in turn, inhibits normal sleep and IL-1–induced sleep (51). Less information is available concerning sleep regulation by other cytokines. However, both tumor necrosis factor and interferon are somnogenic after intracerebroventricular administration (51).

Not only are immune response modifiers capable of altering sleep, but it is also possible that sleep processes and sleep–wake activity may mediate changes of cellular immunity, possibly by producing changes in the nocturnal secretion of immune response modifiers such as interleukins. Indeed, Moldofsky et al. (58) have reported a temporal association between the onset of slow-wave sleep and the secretion of IL-1 and IL-2, two lymphokines that are known to stimulate or activate NK cells. Other data in normal controls have shown that sleep deprivation alters immunity. Palmblad et al. (63) found that 48 hr of sleep deprivation reduced lymphocyte proliferative responses to phytohemagglutinin as compared to baseline levels, and Moldofsky and colleagues (58) found a decline in NK activity following 40 hr of wakefulness. Finally, our group has shown that even modest amounts of sleep disturbance, such as either early (sleep time 3 a.m. to 7 a.m.) or late-night (sleep time 11 p.m. to 3 a.m.) partial sleep deprivation produces a 30% to 50% transient decrease in NK activity.

The effects of sleep deprivation on immune function suggest that insomnia and sleep disturbance, which occur during life stress and depression may be associated with depression-related alterations of immunity. To test whether sleep disturbance accounts for the reliable association between depressive symptoms and reduced immunity, correlational analyses have shown that insomnia is negatively correlated with NK activity in bereaved women, individuals undergoing other severe life stress (35), patients with major depression (40), and patients comorbid for depression and alcoholism (35). Together, these data are consistent with the hypothesis that specific symptom profiles contribute to the reduction of NK activity found in depression, and further implicate the severity of insomnia in depression-related suppression of NK activity.

Insomnia or subjective sleep disturbance is characterized objectively by disturbances of several sleep EEG continuity measures including increased sleep latency and decreased total sleep time and sleep efficiency. To extend the correlational findings between insomnia and NK activity, Irwin and colleagues (37) predicted that EEG measures of sleep continuity would also be associated with NK activity. Indeed, in depressed patients, EEG measures of total sleep time, sleep efficiency, duration of non-REM sleep and Stage 2 sleep were positively correlated with lytic activity. Furthermore, these relationships between sleep amounts and continuity measures were independent of the presence of a mood disorder because similar correlations between lytic

activity and total sleep time, sleep efficiency, and duration of non-REM sleep were found in nondepressed control subjects. It appears that sleep amounts and/or quality, whether assessed by objective or subjective methods, are associated with immune function.

Further studies are needed to examine the mechanisms underlying an association between sleep disturbance and reduced NK activity in depression, although sleep-related changes in lymphokines or neuroendocrine signals are hypothesized. For example, a decrease in amounts of slow-wave sleep occurs in depression, and Moldofsky et al. (58) have reported a temporal association between the onset of slow-wave sleep and the secretion of IL-1 and IL-2, two lymphokines that are known to stimulate or activate NK cells. Alternatively, sleep is associated with decreased sympathetic outflow, and disturbance of total sleep time or loss of sleep efficiency has been proposed to elevate sympathetic activity and in turn reduce cellular immunity.

Activity

Changes in physical activity in depressed patients might also contribute to the reduction of cellular immunity. In our laboratory, assessment of psychomotor retardation and/or agitation by the Hamilton Depression Scale has shown that ratings of retardation negatively correlate with values of NK activity, whereas agitation is positively associated with lymphocyte proliferative responses (17). Separate from the study of depressed patients, a reduction of physical activity such as induced bed rest or immobilization in humans has been found to produce a reduction of immune function as measured by decreased antibody response to specific antigens. Conversely, acute physical exercise sharply increases NK activity (60) as well as circulating levels of interferon and IL-1, and exercise training of sedentary subjects also yields an increase in basal levels of cytotoxicity as compared to nonexercise controls. Finally, given the possible role of NK cells in immune surveillance, it is of interest that increased levels of physical activity are associated with a decrease in the incidence of certain tumors in animals.

Neuroendocrine-immune Interactions

This section focuses on those neuroendocrine hormones that are altered in depression and/or hypothesized to have a role in depression-related immune changes. Anatomical and in vitro evidence will be reviewed that indicates a direct influence of the hormone on immune cells. Discussion of the in vivo regulation of immune function will then be described with a review of clinical studies that have evaluated the relationship between regulation of neuroendocrine function and immunity in depression (see also Chapter 68, Chapter 69, and Chapter 73).

Adrenal Axis

The secretion of corticosteroid has long been considered as the mechanism of stress-induced and/or depression-related suppression of immune function (14, 59). Specific intracytoplasmic corticosteroid receptors have been identified in normal human lymphocytes, and these receptors bind corticosteroids and appear to play a role in the regulation of cellular function through modulation of cyclic adenosine monophosphate (cAMP) levels. Indeed, in vitro studies have demonstrated that glucocorticoids inhibit IL-1 and IL-2 production at the level of cytokine gene transcription with resulting suppression of lymphocyte responses to mitogenic stimulation and NK cell activity (antibody-dependent cytotoxicity is relatively refractory to glucocorticoids) (14).

Glucocorticoids also have numerous in vivo effects on immune function. Serum levels of IgG,

IgA, and IgM are suppressed by pharmacological doses of glucocorticoids in vivo (14). In addition, glucocorticoids affect the distribution of circulating lymphocytes, potently inhibit cellular cytotoxicity, and suppress mitogen-induced T-cell proliferation in vivo (14). B-cell proliferation is relatively resistant to glucocorticoids. Together these data demonstrate that glucocorticoids pharmacologically modulate cellular and humoral immune responses.

The role of in vivo physiological elevations of glucocorticoids in mediating alterations of immune function following stress has not been as conclusively demonstrated as the pharmacological effects of glucocorticoids. For example, in rats, acute administration of either forced immobilization (24), audiogenic stress, or isolation produces an activation of adrenal steroid secretion but does not alter cellular immune function. Likewise with repeated exposure to the stressor, pituitary adrenal activation is dissociated from a reduction in cytotoxicity. Furthermore, stress-induced suppression of lymphocyte function and/or NK activity following inescapable aversive stress occurs in either adrenalectomized or hypophysectomized animals (46). Finally, antagonism of stress-induced activation of the pituitary adrenal axis by the peripheral preadministration of an antiserum to corticotropin-releasing hormone (CRH) fails to alter stress-induced suppression of NK activity even though the release of ACTH and corticosterone is inhibited (41).

Consistent with these animal studies, clinical research has found no relationship between adrenocortical activity and immunity in depressed patients and in stressed persons. In depressed patients, decreased lymphocyte responses to mitogens were not correlated with circulating levels of adrenal cortical hormones (16, 65) increased excretion rates of urinary free cortisol (49) or dexamethasone nonsuppression. Furthermore, in bereavement in which a reduction of NK activity has been demonstrated, we have found that this immunological change occurs even in subjects who had plasma cortisol levels comparable to control subjects. Miller and colleagues (57) also found no association between hypercortisolemia and NK activity in depressed patients. In contrast with these findings, Maes et al. found that baseline cortisol, urinary free cortisol, and postdexamethasone β-endorphin values explained up to 45% of the variance in lymphocyte proliferation, although in these studies lymphocyte function was inversely related to baseline cortisol but positively correlated with urinary free cortisol excretion.

It is alternatively hypothesized that lymphocytes of depressed patients may be even less sensitive to the effect of glucocorticoids (53). Following chronic exposure to glucocorticoids, adrenal steroid receptors expressed in immune tissues exhibit down regulation. Furthermore, dexamethasone-induced inhibition of T-cell blastogenesis is inversely correlated with plasma concentrations of cortisol (77), suggesting the possibility that elevated activity of the adrenal axis down-regulates the lymphocyte glucocorticoid receptor axis in depressed patients and decreases the sensitivity of lymphocytes to pharmacological as well as physiological concentrations of glucocorticoids.

Opioid Peptides

Pharmacological evidence has shown that opiate receptors are located on lymphocytes and macrophages, although few studies have reported on the saturability or stereospecificity of these binding sites (68). Nevertheless, opioid binding on immune cells is suggested by the finding that nanomolar concentrations of opiates affects active rosetting of human T lymphocytes. Morphine inhibits rosetting, whereas *met*-enkephalin stimulates it and both effects are blocked by naloxone.

Using radiolabeling techniques, specific opioid binding on human phagocytic leukocytes (19), platelets, and lymphocytes has been demonstrated. Finally, Carr et al. (6) utilized antibodies to recognize opiate receptors on lymphocytes.

In vitro studies have found that opioid agonists alter a number of immune functions with both inhibitory and immunoenhancing effects described. Johnson et al. (43) found that α-endorphin, *met*-enkephalin, and *leu*enkephalin (all with approximately equal potency) decrease the proliferation and antibody production of splenocytes in the plaque-forming assay, an effect that is blocked by naloxone. Confirming these results, (*des-tyr*)-β-endorphin is also active in suppressing the plaque-forming cell response. Proliferation induced by PHA is affected by opioid peptides, although both stimulatory and inhibitory effects have been reported. Opioid agonist enhancement of mitogen-induced lymphocyte proliferation and IL-2 production are not blocked by the specific opiate antagonist naloxone. Also, NK activity is modulated by opiate agonists; very low concentrations of β-endorphin (10 fM) increase NK cell activity, an effect that is naloxone reversible. Interestingly, β-endorphin induced change of NK activity has been reported to have an inverted U-shaped dose–response curve, and enkephalins and selective opiate agonists also show bidirectional effects on human NK activity; subjects with "low" (below the median) NK activity show stimulation with enkephalin whereas the cytolytic activity of cells from the "high" group is inhibited by similar doses of enkephalin. Recent evidence suggested that endorphins modulate NK activity through the (6–9) amino acid region, that is, the α-helix portion of β-endorphin.

Peripheral administration of opioid agonists in vivo also alters cellular immune function as reflected by suppression of mitogen-induced lymphocyte proliferation, in vivo lymphocyte proliferation in a graft–host reaction (5), production of IL-2 and interferon, delayed-type hypersensitivity response (5), and NK activity. In contrast, induction of humoral immune responses to bacterial or viral antigens is not altered by chronic morphine.

The suppressive effects of peripherally administered opioid agonists has been proposed to be due to an indirect influence of opiates by CNS activation of either the hypothalamic-pituitary-adrenal (HPA) axis or the sympathetic nervous system. For example, the suppressive effects of morphine on the graft–host assay are less pronounced in adrenalectomized mice (5) and acute morphine suppression of NK activity is mediated by both α- and β-adrenergic pathways (6).

Nevertheless, direct peripheral effects of opioid agonists on the immune system are also possible, because opioid agonists bind on specific receptors on cells of the immune system. Indeed, experimental and correlative evidence suggest that peripheral increases of endogenous circulating concentrations of β-endorphin stimulate NK activity, similar to the findings generated in vitro. In the rat, release of β-endorphin into the plasma following acute exposure to forced immobilization are correlated with immediate, poststress *increases* of splenic NK activity. Likewise, clinical research has found exercise-induced enhancement of NK cytotoxicity is completely antagonized by the preadministration of the opiate antagonist naloxone (22). Finally, plasma levels of β-endorphin are *positively* correlated with NK activity in depressed patients (15).

Adrenocorticotropic Hormone

A receptor for ACTH has been identified on lymphocytes, also raising the possibility that some aspects of altered lymphocyte function in depression are regulated by this peptide. In vitro studies

have shown that ACTH inhibits the antibody response at an early state in the antibody response (43). In regards to cellular immunity, the effects of ACTH administered in vitro and in vivo on NK cell function have been evaluated. Exogenous, peripheral administration of ACTH at physiological concentrations increases both NK activity and IL-2 stimulated NK cytotoxicity compared to levels in saline controls. Because in vitro doses of ACTH have no effect on NK activity, the action of this peptide in vivo is likely by an indirect mechanism, that is, alteration of leukocyte traffic or suppression of central CRH. In depressed patients, no association between plasma concentrations of ACTH and lymphocyte proliferation has been demonstrated, although the effects of ACTH on NK activity has not yet been evaluated.

Prolactin

A direct involvement of prolactin in the regulation of the immune system has been proposed. Prolactin receptors have been found on T and B lymphocytes, and prolactin induces a marked enhancement of NK activity. In hypophysectomized rats, as well as animals treated with bromocriptine, a dopamine agonist drug that inhibits prolactin secretion, impairment of both humoral and cell-mediated immune response has been demonstrated that can be reversed by treatment with exogenous, physiological doses of prolactin (3). In addition, antibodies to prolactin have been reported to inhibit lymphocyte proliferation (28). Preliminary observations by Darko and colleagues (16) have found that plasma levels of prolactin positively correlated with T-cell mitogen proliferation, although Clodi et al. (7) failed to show any immune alteration as reflected by serum concentrations of immunoglobulin, IL-1, soluble IL-2 receptor, lymphocyte subsets, and NK activity in patients with prolactinomas. Possibly chronic prolactin elevations lead to adaptive changes such that the immunomodulatory effects of prolactin are abolished.

Growth Hormone

Growth hormone is a polypeptide that plays a pivotal role in growth and development. In addition to its endocrine and metabolic effects, growth hormone is likely to have significant effects on the immune system, particularly T-cell development. In growth hormone-deficient mouse strains, hematopoietic, and B-cell progenitor deficiencies, marked atrophy of the cortical region of the thymus and an impairment of cell-mediated immunity have been found that can be reversed by treatment with growth hormone. In contrast to these observations, Cross and colleagues (11) indicated that hypopituitary mice lag behind heterozygous littermates with respect to development of immunocompetence but that normal immune responsiveness does fully develop. Exploratory clinical studies in depression have failed to identify any relationship between growth hormone and immune function (16), although the numbers of depressed subjects studied is small.

Serotonin

Substantial evidence has demonstrated that serotonergic systems are altered in depression, and recent in vitro data have shown that serotonin modulates lymphocyte proliferation and NK activity. For example, serotonin suppresses lymphocyte response to PHA in vitro, whereas the addition of serotonin to mononuclear cells enriched for NK activity induces a twofold dose-dependent enhancement of NK activity, similar to the stimulation induced by IL-2 (30). The induction of NK activity is monocyte dependent and is likely mediated by specific binding of serotonin at the 5-HT_1 receptor on the monocyte; the enhancing properties of serotonin are mimicked by the 5-HT_1 specific receptor agonist 8-OH DPAT and completely antagonized by the

serotonin receptor antagonist cyproheptadine. Clinical studies have not yet characterized whether expression and regulation of the 5-HT_{1A} receptor on monocytes is altered in depressed patients, and it is not known whether monocyte 5-HT_{1A} activity has a role in the reduction of NK activity in depression (see also Chapter 39).

Autonomic Nervous System

Anatomical studies have revealed an extensive presence of autonomic fibers in both primary and secondary lymphoid organs (21), innervating both the vasculature and the parenchyma of the tissues and serving as one pathway for communication from the brain to cells of the immune system. Immunohistochemical studies of splenic tissue have demonstrated that nervous fibers containing norepinephrine, substance P or neuropeptide Y branch into the parenchyma (21) where lymphocytes (primarily T cells) reside. These postganglionic sympathetic fibers are not only adjacent to T cells but, at the electron microscopic level, end in synapticlike contacts with lymphocytes in the spleen (21).

In the adult animal, norepinephrine appears to act as a neurotransmitter in the spleen. Norepinephrine is likely released within the spleen; early studies found that splenic nerve stimulation yields a release of norepinephrine. Furthermore, in vivo dialysis techniques documented a 1-μM concentration of norepinephrine in the rat spleen (21); a concentration that is more than 100-fold higher than that in blood suggesting local release of norepinephrine within the spleen.

Lymphocytes have been found to receive signals from the sympathetic neurons by adrenoreceptor binding of norepinephrine, epinephrine, and dopamine. These β-receptors are linked to adenylate cyclase and have a functional role in the modulation of cellular immunity. In vitro incubation of lymphocytes with varying concentrations of either norepinephrine or epinephrine decreases NK activity and mitogenic responses, an effect that is reversed by a β-receptor antagonist. Additional in vitro studies have found that neuropeptide Y, a sympathetic neurotransmitter present in peripheral sympathetic nerves, also acts to inhibit NK activity (61).

In vivo studies have shown that the sympathetic nervous system has a role in the modulation of immune function. Either surgical denervation of the spleen or chemical sympathectomy using the neurotoxin 6-hydroxydopamine produces an augmented antibody response to thymus-dependent antigens such as SRBCs, an enhanced plaque-forming cell response to thymus-independent antigens, increased macrophage phagocytosis, and enhanced T- and B-cell responsiveness to mitogen stimulation that is strain specific. Conversely, infusion of adrenergic agonists such as isoproterenol in rats and in humans results in a down-regulation of β-adrenergic receptors in circulating mononuclear cells and a dose-dependent, transient decrement in mitogen responses that is likely mediated by a redistribution of circulating lymphocyte subpopulations via β_2 adrenergic mechanisms (60). In addition to sympathetic-induced alterations of nonspecific immune function, sympathetic neurotransmitters also suppress antigen-specific lymphocyte proliferation by inhibiting macrophage antigen processing and presentation and, indirectly, T-helper responses.

Acute, stress-induced elevations of sympathetic activity is one pathway that likely plays a role in stress-induced suppression of cellular and humoral immune responses. For example, in animals exposed to aversive stress or central doses of neuropeptides such as CRH that activate

sympathetic outflow, suppression of cellular immunity or in vivo specific antibody responses is completely antagonized by either autonomic blockade, chemical sympathectomy, or β-receptor antagonism (13, 36).

Chronic elevation of sympathetic tone has also been found to mediate a reduction of immunity (34). For example, in animals, induction of a chronic hyperadrenergic state (experimental congestive heart failure or 2-week infusion of the β-agonist isoproterenol) reduces NK activity, in vivo antibody responses, and lymphocyte proliferation. Similar to findings following acute stress, the immunosuppressive effect of chronic increased sympathetic tone is completely antagonized by β-receptor blockade. In humans, chronic elevated sympathetic tone, as reflected by circulating concentrations of neuropeptide Y, may also contribute to the modulation of immune function during severe life stress and depression (34). Neuropeptide Y is present in peripheral sympathetic nerves and is released following emotional stress potentiating the effects of vasoactive catecholamines and other pressor substances. Irwin et al. (34) have shown that plasma concentrations of neuropeptide Y are elevated in depressed patients as well as in aged individuals and persons undergoing severe Alzheimer caregiver stress. Furthermore, activation of the sympathetic nervous system and release of neuropeptide Y is associated with a reduction of natural cytotoxicity in depression and life stress. Additional findings also support the hypothesis that elevated sympathetic activity in depression is associated with immune alterations. In depressed patients, excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG) has been used as an index of total body noradrenergic turnover or sympathetic activity, and MHPG excretion was inversely related with lymphocyte proliferative responses in depressed patients (54). Together, these data suggest that elevated sympathetic tone in patients with major depressive disorder and/or in persons undergoing life stress is inversely correlated with cellular immune function.

Central Nervous System

Central Lesion Studies

Much evidence exists that the brain coordinates autonomic nervous system and neuroendocrine outflow, which has been shown in separate studies to mediate immune function. To evaluate the role of the brain in the neural modulation of immunity, early experiments placed electrolytic or other destructive lesions in the CNS and demonstrated alterations in lymphoid tissue architecture, either impairment or enhancement of lymphoid cell activation, impairment of delayed-type hypersensitivity, and suppression of NK cell activity. Furthermore, hemispheric lateralization of immune control is evident, since partial ablation of the left hemisphere cortex, which results in a relative right-sided activation, produces significant decreases in the number of T lymphocytes and NK activity. Comparable lesions in the right cortex either have no effect or increase immune responses, although some investigations have found that right as compared to left decortication of mice produced a greater reduction of natural cytotoxic activity (2). In an effort to extend these preclinical observations of hemispheric lateralization of immune modulation, patterns of hemispheric activation in the frontal scalp region have been related to immune responses in women who show extreme differences in the asymmetry of frontal cortex activation (44). Consistent with animal studies, women with extreme right frontal activation had lower levels of NK activity than did left frontally activated individuals. Measure of lymphocyte proliferation and T-cell subsets did not differ.

Central Catecholamines

Additional studies have further evaluated the role of the brain in modulation of immunity and tested whether central catecholamine activity is associated with immune function. Injection of the neurotoxin 6-hydroxydopamine into the cisterna magna depletes CNS catecholamines and impairs the primary antibody immune response by inducing T-suppressor-cell activity (12). Further studies have evaluated the relationship between stress-induced central catecholamine alterations and changes in splenic NK activity and found positive correlations between mesocortical dopamine and dihydroxyphenylacetic acid (DOPAC) and NK activity but no relation between catecholamines and NK activity, possibly due to the limited number of brain regions, the large tissue samples assayed, and assay of catabolic products rather than in vivo release.

Central Opiates

Opiate-induced modulation of NK cytotoxicity is mediated in part by the CNS. Injection of methionine enkephalin directly into the cisterna magna results in an enhancement of NK cell activity. As well, opiate-mediated pathways in the brain produce polyinosinic:polycytidilic acid (poly I:C) conditioned-enhancement of NK activity. In contrast with these observations, NK activity is suppressed when a dose of morphine is given into the cerebral ventricle, an effect that is likely mediated by central opiate receptors since the effective central dose is a thousand times smaller than that required when given systemically. Moreover, peripheral administration of low doses of a morphine analog (one that does not cross the blood–brain barrier to enter the CNS) is not effective in altering NK activity. Finally, intracerebral infusion of morphine into the periaqueductal gray, but not other brain regions, produces a suppression of splenic NK activity (74). Use of selective μ, κ, and δ agonists microinjected into the lateral ventricle have further shown that central opiate-induced suppression of NK activity is mediated primarily through μ-type opioid receptors.

Central Interleukin-1β

Interleukin-1, a cytokine originally detected in the macrophage, has been preliminarily shown to be increased in the peripheral circulation of depressed patients (55). In addition to its biological effects peripherally, this cytokine when introduced into the brain induces slow-wave sleep, releases hypothalamic CRH, and produces increased circulating concentrations of ACTH. Because IL-1 plays a crucial role in regulating a number of immunological responses, Sundar et al. (73) and Brown et al. (4) have investigated whether central administration of IL-1 would affect immunity and demonstrated that central doses of IL-1 (3.1 to 12.4 fmol) rapidly reduces ex vivo cellular immune responses such as NK cell activity, lymphocyte proliferative response to PHA, IL-2 production, and macrophage IL-1 secretion. Importantly, the effects of central IL-1 on immune function are blocked by the administration of an antibody to CRH, indicating that IL-1β likely mediates immunosuppression by central release of CRH (64, 73). Consequently, the pathways by which IL-1 regulates peripheral immune function are similar to mechanisms involved in central CRH suppression of immunity. Administration of a ganglionic blocker abrogates the effects of IL-1 (73) similar to observations that autonomic and sympathetic nervous mechanisms mediate CRH-induced suppression of NK activity (36).

Central Corticotropin Releasing Hormone

Depressed patients show elevated concentrations of cerebrospinal fluid CRH and this

neuropeptide has been postulated to be a physiological CNS regulator that integrates biological responses to stress. Relevant to depression-related reduction of cellular immunity, central CRH has indeed been found to have a physiological role in mediating stress-induced suppression of immunity (41, 42). Antiserum to CRH when given centrally into the lateral ventricle prior to foot shock stress completely abrogated stress-induced suppression of cellular immunity. In contrast, peripheral administration of antiserum to CRH failed to attenuate suppression of immunity following foot shock even though stress-induced release of ACTH and corticosterone was significantly antagonized. Further observations have supported the hypothesis that brain CRH coordinates immune function. Central administration of CRH, at doses known to act at extrahypothalamic sites to induce an increase in the firing rate of the locus coeruleus and activate autonomic outflow, produces a dose-dependent suppression of NK activity, in vivo specific antibody responses (33), and lymphocyte proliferation. In contrast, peripheral administration of CRH fails to suppress either cellular or humoral immune responses, even though such doses activate the pituitary adrenal axis. Finally, coadministration of the CRH antagonists immediately prior to a central dose of CRH has been found to antagonize central CRH-induced suppression of immune function, indicating that CRH coordinates reduction of immunity in a receptor-mediated manner in the brain, possibly at stress sites such as the locus coeruleus or central nucleus of the amygdala (33).

As reviewed above, considerable evidence has shown that noradrenergic projections to lymphoid tissue have a role in the modulation of immune function, and central CRH is crucial in coordinating sympathetic outflow. Neuroimmunological data have demonstrated that CRH activation of the sympathetic nervous system is essential in mediating the link between the brain and immune function. Either ganglionic blockade, sympathetic denervation, or β-adrenergic receptor antagonism completely abrogates CRH-induced suppression of cellular immunity (36). Again, CRH-induced modulation of immune function is dissociated from circulating levels of ACTH and corticosterone.

The health implications of these data are not known. However, it has recently been proposed that older depressed persons who have been shown to have increased morbidity and mortality are also more vulnerable to depression-related immune decline. To test the hypothesis of increased vulnerability of the aged to stress- related immune decline, CRH has been used as a neuropeptide probe and sympathetic and immune responses of aged rats to central CRH were compared to responses in young animals. Central CRH has been found to induce an exaggerated sympathetic activation and a greater suppression of NK activity in the aged rats as compared to responses in young animals, suggesting an increased responsivity or vulnerability of the aged to stress-induced immune suppression. Further data showing that central CRH modulates in vivo specific antibody responses (33) suggest that, under conditions in which CRH is released, such as stress or possibly depression in humans, the immune response may be down-regulated, leading to a delay in the synthesis of adequate levels of antibodies and failure of the immunological protection of the host to an infectious agent. Indeed, Glaser et al. (27) have shown that the immunological defense that is assumed to follow a vaccination may not be fully operative if the immunization occurs during times of stress. Consistent with these findings that CRH-induced immune modulation may have health implications, decreases in the central secretion of CRH have been associated with pathological increases of immune function. Lewis rats show a defect in CNS biosynthesis of CRH and these rats exhibit an increased susceptibility to streptococcal cell-wall-induced inflammatory arthritis (72).

Whereas endogenous brain CRH has been found to have a role in the in vivo suppression of immunity, additional data have suggested that CRH might also have local direct effects on immune processes, interacting with immune cells by paracrine mechanisms to provide a peripheral counterbalance in the regulation of the immune response. For example, CRH is contained in T lymphocytes, and PHA stimulation induces an increase in CRH mRNA levels. In vitro studies have reported both stimulatory (69) and inhibitory effects of CRH on cellular immune function, whereas peripheral injections of CRH have been found to promote several immune functions. For example, peripheral in vivo administration of CRH binds at receptors on macrophage (75), enhances macrophage secretion of IL-1, and increases secretion of β-endorphin from lymphocytes leading to stimulation of the antibody response when the control response was low (29). Karalis et al. (45) have also shown that CRH produced by immune cells has autocrine inflammatory action, such that in vivo administration of antiserum to CRH caused suppression of both inflammatory exudate volume and cell concentration. These data regarding the peripheral direct effect of CRH on immune function suggest that CRH is capable of acting on immune cells in a manner that is antithetical to that of hypothalamic CRH (see also Chapter 49Corticotropin-Releasing Factor: Physiology, Pharmacology, and Role in Central Nervous System and Immune Disorders and The Role of Acetylcholine Mechanisms in Mood Disorders).

HEALTH IMPLICATIONS OF DEPRESSION-RELATED IMMUNE ALTERATIONS

The clinical importance of altered cellular immunity in depressed patients is not yet known. Although substantial evidence has been found for an association between stress or depression and increased illness (10), the association between psychological stress and immune-related disease remains limited to several studies. In regard to infectious illness, Cohen et al. (9) have recently shown that psychological stress was associated in a dose–response manner with an increased risk of acute infectious respiratory illness. Importantly, this association was due to increased rates of infection rather than to an increased frequency of symptoms after infection. Measures of cellular immune function were not obtained in this study. Other studies, but not all, have found that stressful events increase the risk of verified acute respiratory illness. Depressed patients have been found to show a higher incidence and higher titers of herpes simplex virus antibodies, but again no study has yet delineated a causal chain showing that severe life stress or a particular psychological state such as depression produced an immunological response, which then resulted in an altered clinical outcome.

The findings of increased cancer incidence in clinically depressed patients are consistent with a "null or weak relationship" (25). Although several prospective follow-up studies have demonstrated a relationship between mood disorders and clinical depression and increased cancer morbidity and mortality particularly in male patients over age 40 who had primary diagnoses of mood disorders, small case numbers indicate that these results should be cautiously interpreted. Likewise, epidemiological studies on the role of depression in cancer have been inconclusive. Depressed mood as measured by the Minnesota Multiphasic Personality Inventory depression scale was found to be a significant risk factor for cancer incidence and mortality over 20 years of follow up. However, subsequent studies have failed to replicate the observation that depression is associated with an increase in the relative risk of cancer. However, other factors such as smoking or alcohol use have been proposed to interact with depressive symptoms to increase the risk of cancer, and an increased risk of cancer has been reported in persons with depressed mood who

are smokers when compared with persons who are never smokers without depressed mood. Furthermore, there was a dose–response effect of cigarette smoking on the depressed mood–cancer relationship in the smokers.

The promise of recent investigations has suggested that psychiatric interventions that reduce psychological distress may have beneficial effects on cancer survival. Spiegel et al. (70) found that group psychotherapy of breast cancer patients improved quality of life and extended life expectancy for patients with metastatic breast cancer, and Fawzy et al. (20) showed that other types of cancer patients improve both psychologically as well as immunologically with psychosocial group therapy. The psychoneuroimmunological link between depression and psychological stress and the outcome of immune-related medical disorders will be refined by further research that addresses the role of depressive symptoms, such as sleep disturbance, in the modulation of immunity and defines the neurobiological paths that underlie the interaction between the brain, behavior, and immunity.