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BIDIRECTIONAL INTERACTION BETWEEN THE CENTRAL NERVOUS SYSTEM AND THE IMMUNE SYSTEM

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I. INTRODUCTION

An important question to ask when preparing an article for immunologists that discusses an interaction between the brain and the immune system relates to whether an immunologist should be concerned about this interaction. We hope that the information that we have included will be convincing in establishing the existence of such an interrelationship. Although the implications regarding disease susceptibility are less clear, we present convincing examples in animals and humans that indicate that immune reactivity can be influenced by psychosocial factors, even extending to the conditions used for the housing of experimental animals. In addition, we indicate some possible mechanisms by which the central nervous system (CNS) can alter immune system function. Finally, we provide information suggesting that the brain is not an immunologically privileged site but rather should be viewed as any other organ of the body with respect to susceptibility to immunologic attack.

There is, as yet, no agreement as to what to call this newly emerging field of scientific investigation. Several proposed terms include neuroimmunomodulation, behavioral immunology, or psychoneuroimmunology. Whatever term is finally adopted, it is important that it implies the existence of bidirectional interaction between the CNS and immune systems. In this review we use the term "psychoneuroimmunology".

The primary purpose of the immune system is to provide protection from infectious agents. As an example, children born with congenital immune deficiency diseases and adults who acquire immune deficiencies in later life are at an increased risk of developing an infection with bacteria, viruses, fungi, or protozoan organisms.[1]

Deliberate suppression of the immune system is produced in individuals receiving an organ transplant. While the immunologic suppression enhances the survival of the transplant,

the patients are at an increased risk of developing infections and malignancy. Thus, a normal functioning immune system is critical for the maintenance of health and anything that alters immune system function may alter susceptibility to disease.

Extensive epidemiologic and experimental information exists indicating that a stressor is capable of producing alterations in immunologic function or disease susceptibility (Sections V.B, V.C, and V.D). Anecdotely, many individuals believe that susceptibility to infectious disease increases when experiencing stress. Although increased susceptibility to infections in individuals who are otherwise healthy is hard to document, the onset or exacerbation of autoimmune disease can be more accurately determined. In this respect, numerous studies have shown that the onset and relapse of autoimmune disease is frequently associated with a stressor.[2]

Immunologists have accepted the etiology of insulin-dependent diabetes as being due to an immunologic reaction against the insulin-producing cells of the pancreas. Thus, the exquisite specificity of the immune system with respect to its ability to attack specialized cells of the body with a resultant physiologic change is apparent. If the brain can be a target of the immune system, we must consider that a similar exquisite specificity exists and that different parts of the brain may be attacked, leading to discrete clinical syndromes. If this hypothesis can eventually be proven, a radical new approach to understanding mental disorders may be provided.

The association of altered disease susceptibility when one is experiencing stress suggests that the CNS may be capable of influencing the function of the immune system. The mechanism of such interaction may be by direct innervation of lymphatic tissue or by the release of hormones from the brain that bind to membrane receptors on immunologically active cells.

II. WHAT IS STRESS

A working definition of stress is needed. The term "stress" is commonly used in different ways:

1. As a severe environmental condition
2. As an appraisal of an environmental situation
3. As a specific set of biological responses to a situation
4. As a specific set of behavioral responses to a situation
5. As a description of a situation in which environmental demands exceed coping resources[3-5]

This conceptual confusion, however, is not directly reflected in studies of stress and immunity. For the most part both animal and human studies view severe environmental conditions as stressful. Examples of manipulations in animal studies include electric shock, crowding, changing housing conditions, and turntable rotation. Examples of measures used in human studies include recent bereavement, impending exams, and recent stressful life events. Human studies have also included behavioral response measures of stress, including self-reports of anxiety, depression, or general psychological distress. For the purpose of this review, we will make the simplistic assumption that all of these manipulations and measures are tapping the same underlying process. We do this with the awareness that some of the inconsistencies in the literature we address are partly or wholly attributable to inconsistent definitions and conceptualizations of the stress concept.

Stress may be linked to disease through direct CNS-immune links or through CNS-neuroendocrine-immune pathways. However, we do not need to assume a role of the CNS in order for stress to influence disease susceptibility through its effects on behavior. For

example, persons under stress tend to smoke more, drink more alcohol, eat poorly, and sleep less. All of these factors put them under greater risk for disease by either influencing immunity or other biological processes.

It is well known that the CNS endocrine system interaction is bidirectional. When the hormone products of endocrine organs, such as the thyroid or adrenals, increase in concentration, the pituitary responds by decreasing the production of peptides that stimulate the endocrine tissue to release hormones. A bidirectional interaction between the CNS and immune systems will be important to establish in order to strengthen the analogy that both the immune and endocrine systems have similar interrelationships with the CNS. Indeed, cells of the immune system can produce hormones that are usually associated with the endocrine system.[6] Pathways probably exist where an activated lymphoid cell produces a hormone that acts on the CNS and induces hormone production that downregulates the activated immune system.[7]

III. PATHWAYS LINKING THE CNS WITH IMMUNE SYSTEM

An important question involves the pathways and mechanisms by which the CNS can influence the functional activity of the immune system. Possibilities include the release of hormones from the brain. The hormones then circulate through the bloodstream and alter the functional activity of immune cells. Examples of this mechanism include hormones released by the pituitary that enter the circulation and affect tissue, such as the thyroid or adrenal glands. Alternatively, hormones released from the brain may affect the release of other hormones from endocrine tissue that could modify immune function. An example of this would be cortisol release from the adrenal gland arising from ACTH production by the pituitary.

Examples of hormones produced by the brain or in response to elevated brain hormone levels have been reported to produce either increased or decreased immune function. The concentration of a substance, the presence of multiple other substances that may simultaneously be present, the type of lymphoid cell population being evaluated, and the number of specific receptors on different cell populations must all be taken into consideration when these studies are evaluated.

A. HORMONE MEDIATORS

Endorphins are a group of endogenous brain polypeptide hormones. It has been found that these naturally occurring peptides can exert opiate-like effects in the brain by interacting with opiate receptors on cell membranes. Enkephalins are pentapeptides that are also classified as endorphins. Both beta-endorphin and enkephalin may bind to lymphocytes and have been found to both enhance and suppress immune function.[8,9] Whether there is enhancement or suppression of immune function may depend on the concentration of the neuropeptide, the cell population being assayed, and the species studied.[10,11]

The effect of the opiate-like hormones on the function of the immune system has been evaluated both *in vitro* (using rodent and human lymphocytes) and *in vivo*. Alpha-endorphin inhibits the primary, *in vitro*, antibody response of human peripheral blood lymphocytes to ovalbumin when present in low concentration (5.0 μg/ml) but does not interfere with the response when present at 10 μg/ml.[12] Alpha-endorphin can also inhibit the *in vitro* antibody response to mouse spleen lymphocytes to sheep erythrocytes.[13] Both leu- and met-enkephalin inhibit the response to sheep erythrocytes, but are less effective than alpha-endorphin. Beta-endorphin, from which alpha-endorphin is split, can decrease the response of human peripheral blood lymphocytes to phytohemagglutinin (PHA) *in vitro* when present at concentrations of 10^{-7} to 10^{-9} *M*.[14] Leu-enkephalin has also been reported to decrease the mitogenic response of human lymphocytes to PHA.[15]

Some reports indicate that enhancement of *in vitro* reactivity of lymphocytes occurs upon incubation with hormones such as beta-endorphin or leu- and met-enkephalin. Human, mouse, and rat lymphocytes have been studied. Responses such as increased proliferation to PHA, enhancement of the plaque-forming cell response to ovalbumin, and increased natural killer (NK) cell activity have all been reported. However, the mechanism of enhancement or suppression of immune reactivity by brain-related hormones has not been well characterized. It is known, however, that beta-endorphin does not work by binding to the interleukin-2 (IL-2) receptor.[16,17]

Other hormones, such as adrenal steroids, have been implicated in immunomodulation. With respect to the effect of adrenal steroids on immunologic function, there are a variety of conflicting results that have been published. Part of this problem may be due to the varying response of different species to the effect of adrenal steroids. Some species are extremely sensitive to corticosteroids (e.g., rat, mouse, and rabbit), while other species are relatively resistant to these effects (e.g., human, monkey, and guinea pig). Species that are sensitive to corticosteroids have lymphocytes that are lysed by pharmacological levels of the hormone. Thus, species must be considered in any study.

Clinical observations of increased susceptibility to infection with elevated adrenal hormone levels have been extensive.[18-21] A variety of experimental approaches that use a stressor and quantitate adrenal cortical hormone production indicate that elevated levels of the hormone produce immunologic suppression. In addition, *in vitro* studies and *in vivo* situations where adrenal hormone levels are experimentally elevated indicate the immunosuppressive effects of adrenal hormones. These studies showed impairment of neutrophil migration and lymphocyte function.[22-24] Moreover, adrenal cortical steroid hormones have been found to increase the absolute number of neutrophils in the peripheral blood of a patient and decrease the number of lymphocytes.[25]

Although there is substantial evidence to indicate that adrenal cortical hormones induce suppression of the immune system, the relationship is extremely complex. The influence of adrenal cortical hormones on the immune system seems to depend upon the species, type of assay, and concentration of adrenal cortical hormone as well as the subpopulations of lymphocytes assayed. Thus, more research is needed to clarify the relationship among the adrenal corticosteroids and the immune system.[25-29]

Several other hormones may be involved in the regulation of the immune system. The possibility that prostaglandin E2 participates in the immunological suppression associated with stress has been suggested.[17]

Interestingly, there is evidence that lymphocytes produce hormones such as ACTH and endorphin, which may themselves regulate immune function.[7] Thus, there is considerable interaction between the central nervous and immune systems, and possibly even internal control loops of neuropeptides that are produced by the immune system to regulate its function.

B. INNERVATION OF IMMUNE SYSTEM

A possible route of communication between the CNS and the immune system is for nerves to terminate in lymphoid organs with the subsequent release of chemical mediators that could locally alter lymphocyte function. An extensive series of investigations has established that such innervation exists. Direct autonomic innervation by noradrenergic nerves that tend to terminate primarily in regions of lymphoid tissue where T lymphocytes and macrophages predominate has been demonstrated.[30] The noradrenergic nerves of rats arise from the superior mesenteric coeliac ganglion and enter the spleen alongside the splenic artery where the fibers then follow the vasculature to the central arteriolar system of the white pulp. Some of the fibers then enter the parenchyma of the lymphoid nodule around the splenic arterioles. This area is rich in T lymphocytes.[31] Electronmicroscopic studies[32] showed that the nerve terminals actually contacted lymphocytes.

The innervation studies are important as functional changes of the immune system are produced by sympathectomy, either by chemicals or surgery. An enhanced splenic antibody response,[33,34] as well as increased cutaneous delayed hypersensitivity,[35] occur in mice following sympathectomy. These observations suggest that the sympathetic nervous system may suppress the immune system. However, there are other studies that have been reported in which sympathectomy reduces the immune response.[36-38] The strain of animal, the timing of the sympathectomy, the type of antigen, and other stressors to which the animal may be exposed may all influence the immune response following sympathectomy. In addition, lesions of the brain can alter functional activity of the immune system.[39] The pathways involved in this response have not been defined.

In addition to the finding of catecholamines in nerve endings in lymphatic tissue, a variety of other neurohormones are detected in nerve fibers in lymph nodes. Included are neuropeptide Y, vasoactive intestinal polypeptide, and substance P. The release of these peptides within lymphatic tissue may modify immune function if the receptors for the peptides are present on lymphoid cells. The complexity of such immune modulation is obvious if there are peptides present that can augment immune function, while there are also peptides present that can inhibit immune function. It is important that increased efforts be directed toward understanding this complex interaction.

C. THE IMMUNE SYSTEM ALTERING THE FUNCTION OF THE CNS

Interleukin-1 (IL-1) is a product of macrophages and monocytes. Generally, IL-1 is believed to enhance immune system function by stimulating T helper cells to produce IL-2. However, IL-1 is also capable of influencing the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Possible mechanisms for this may involve IL-1 binding directly to cells of the pituitary or IL-1 activating the hypothalamus to release corticotropin-releasing factor (CRF). Both mechanisms may be operative. It has been shown that recombinant human IL-1 can stimulate the secretion of ACTH, luteinizing hormone, growth hormone, and thyroid-stimulating hormone by rat pituitary cells in culture.[40] In addition, IL-1 containing neurons have been found to impinge upon the paraventricular nucleus of the hypothalamus, an area from which the CRF neurons emerge.[41]

There is also some evidence to suggest that the thymus gland produces hormones that can affect the HPA access. Removal of the thymus lowers the serum concentration of ACTH and this effect is reversed by providing thymic extracts.[42] It has also been shown in both nonhuman primates and rodents that a preparation of bovine thymus gland can increase the concentration of circulating ACTH, suggesting that thymus hormones may act upon the pituitary.[43,44] Thus, pathways for regulation of the immune system by the immune system may exist. With a heightened immune response to an antigen, hormonal substances produced by immunologically active cells or in response to thymic hormones may lead to the production of other hormones (cortisol) that would then downregulate the immune system. This mechanism may be important to prevent uncontrolled proliferation of immune cells.

Future research directions will include the determination of receptors for those neurohormones that can bind to lymphoid cells and modify the function of different lymphocyte populations. The role of these receptors in modifying immune function and even having an influence on lymphocyte trafficking must be determined.[45]

It is also important that attention be directed to individual lymphocyte subsets (T-helper lymphocytes, T-suppressor lymphocytes, T-cytotoxic lymphocytes, NK cells, and B lymphocytes) and the effect of more than one neuropeptide binding to a specific lymphocyte. Laboratory conditions can be idealized where the effect of a single peptide can be determined with respect to modifying functional activity. However, it is more likely that multiple peptides are released from nerve endings in lymphatic tissue and the interaction between the peptides determines immune function. Thus, this exciting area of investigation is just beginning to

be explored and a significant advance in our understanding of neuroimmune modulation should be forthcoming.

IV. PSYCHOLOGICAL AND BEHAVIORAL FACTORS IN IMMUNITY

A. STRESS-INDUCED IMMUNOSUPPRESSION IN ANIMALS

Probably the most convincing evidence that indicates that the brain can alter immunological functions is obtained by the use of conditioned responses in experimental animals. In conditioning, an unconditioned stimulus (something that will cause a change and/or is aversive) is temporally coupled to a conditioned stimulus (something that by itself does not produce a change and/or is not aversive). An experimental animal learns to associate the conditioned stimulus with the unconditioned stimulus. When the animal is then presented with the conditioned stimulus it may react as if it is being presented with the unconditioned stimulus.

Pioneering work in this area has been done by Dr. Robert Ader who has paired cyclophosphamide injection (unconditioned stimulus) with saccharin-flavored drinking water (conditioned stimulus). When animals that had been injected with cyclophosphamide and simultaneously given saccharin-flavored drinking water were reexposed to saccharin alone, immunologic suppression occurred.[46] The immune response to sheep erythrocytes as well as an amelioration of the severity of systemic lupus erythematosus in lupus-prone mice has been reported[46] using this paradigm.

Stress in the form of aversive physical stimulation can have a pronounced effect on immune function. For example, it has been reported that intense auditory stimulation for mice decreases the response of splenic lymphocytes to the nonspecific mitogens, Concanavalin-A (Con-A) and lipopolysaccharide.[47] However, our laboratory has been unable to duplicate these findings. Likewise, presentations of electric shock to rats have been shown to decrease the responsiveness of lymphocytes to stimulation by plant lectins,[48] decrease NK cell activity,[10] and increase susceptibility to tumor challenge.[49,50] Although some, but not all, reports agree that physical stressors can suppress immune reactivity, there have been few systematic evaluations of the parameters of the aversive experience or the compartments of the immune system that are affected.

Keller and colleagues[48,51] showed that intense shocks induce a greater reduction in the lymphocyte responsiveness of whole and isolated blood-lymphocyte cultures than do shocks of a weaker intensity. In contrast, their findings also indicated that splenic lymphocytes were not affected by the presentation of many shocks.[48]

Studies conducted in our laboratory have found that the magnitude of suppression of lymphocyte responsiveness induced by electric shock is positively related to the number of shock presentations within a daily session.[52] That research showed a comparably pronounced suppression of mitogenic responsiveness in both the blood and spleen lymphocytes following a single session of shocks. However, the decreased reactivity for the spleen lymphocytes attenuated with repeated sessions of shocks, in contrast to the whole blood lymphocytes, which did not show any attenuation. Our findings, together with those of Keller and colleagues, indicate that initial exposure to electric shock induces suppression of both blood and spleen lymphocytes, and continued exposure will result in attenuation of the suppressive effect, but only for the spleen lymphocytes. These results document that different compartments of the immune system are differentially sensitive to variations in aversive stimulation.

Shavit and colleagues'[10,53] investigation of the mechanisms mediating the immunosuppressive effects of foot shock have suggested that the production of endogenous opiates is important. They demonstrated that intermittent foot shock, which is capable of suppressing NK activity in rat splenocytes, also produces an opioid analgesia. In contrast, continuous

foot shock of equal duration, which does not produce NK suppression, generates a nonopioid form of analgesia. They also reported that the suppression of NK activity was prevented by the administration of the opiate receptor antagonist, naltrexone, prior to foot shock. In support of opiate involvement in immunosuppression, it has also been shown that administration of the exogenous opiate, morphine, can induce a similar suppression of NK activity.[53]

All of the above studies confound the physical and psychological aspects of stressful stimulation, and thus it is difficult to conclude from that research that psychological stress itself modulates immune function, or that similar mechanisms underlie immunomodulation by physical and psychological stressors. However, there is evidence that immune alterations induced by a physical stressor can be modified by psychological factors, such as the animal's training experiences. For example, rats permitted to control electric shocks behaviorally, i.e., by learning to escape them, show far less suppression of lymphocyte reactivity than yoked subjects given the same but inescapable shocks.[54] These experiments suggest that the ability to cope with stress may modify its negative influence on the immune system. It is important to confirm these studies and investigate the effect of coping on a variety of immune parameters.

1. Parametric Evaluation of Stressor-Induced Immune Alteration

Our first investigation[52] was a parametric study of the immunosuppressive effects of different amounts of signaled-shock training, which varied in both the number of signaled shocks per session and the total number of sessions. Thus, rats were given 4, 8, or 16 signaled shocks per day for 1, 3, or 5 d. The signaled-shock trials for all groups occurred on a 4-min variable time (VT) schedule, with each shock (5 s, 1.5 mA) being preceded by a 15-s clicker conditioned stimulus that coterminated with the shock. In addition, the start of training for the 1- and 3-d subjects was delayed so that all groups, including control subjects that had been left undisturbed in their home cages, were sacrificed on the same day.

Both the spleen and a sample of blood from the abdominal aorta were taken from each animal for use in mitogen-stimulation assays. The assays showed that the reactivity of both the spleen and whole blood lymphocytes to Con-A was suppressed for the experimental subjects, as compared with the controls, with the magnitude of that suppression being positively related to the number of signaled shocks per session. However, the suppressed reactivity for the spleen cells diminished with repeated sessions of frequent shocks (8 or 16/d), in contrast to the whole blood lymphocytes that did not show any attenuation of suppression. The mechanism of habituation in the spleen is not yet understood. Possibilities include altered lymphocyte flow patterns or receptor modulation on lymphocyte surfaces.

On the basis of the above findings, we conducted a follow-up study[52] to determine the amount of time that would be required for the subjects to recover from the suppressive effects of their signaled-shock experiences. In that experiment, different groups of rats were given a single session of 16 signaled shocks, as above, and then were left to recover in their home cages for 24, 48, or 96 h prior to their sacrifice for the mitogen stimulation assays. The results of that study showed that the decreased reactivity of the whole blood lymphocytes extended well beyond the immediate period of the signaled-shock trials and took between 48 and 96 h to recover completely. In contrast, the splenic lymphocytes showed complete recovery within 24 h of the shock experiences.

The consistency of our procedures and the reproducibility of our data are apparent in a series of experiments designed to investigate the effect of naltrexone on the suppression of the mitogenic response and suppression of NK cell activity induced by presentations of electric shock. The suppression of NK cell activity was prevented by administration of naltrexone prior to presentation of the shock. However, naltrexone did not prevent the shock-induced suppression of mitogenic responsiveness to Con-A and PHA. These results indicate that stressor-induced immune alterations are mediated by different pathways.[55]

To determine whether the observed stress effects were limited to electric shock, we conducted an assessment of immune function following a single or multiple injection(s) of 2-deoxy-D-glucose (2-DG), a metabolic stressor. The injection of 2-DG, an antimetabolic glucose analog, produces an acute intracellular glucoprivation. Acute glucoprivation is a metabolic condition that shows the physiological hallmarks of a physical stressor.

The results of our investigations[56] showed that a single injection of 2-DG decreased reactivity on both whole blood and spleen lymphocytes in rats, as determined by mitogenic responsiveness to Con-A and PHA. However, whereas the suppressed reactivity for the spleen lymphocytes attenuated with repeated injections, the whole blood lymphocytes did not show attenuation. Mitogen assessment of lymphocytes obtained from the thymus indicated that a single injection did not induce suppressed reactivity, but repeated injections induced a pronounced suppression of responsiveness. Furthermore, assessments of mesentery lymph nodes did not show any effect of 2-DG injections. These results show the same effects as our finding based on electric shock as the stressor. Collectively, the findings indicate that different compartments (blood, spleen and lymph nodes) of the immune system are differentially affected by a stressor.

2. Conditioned Immune Alteration

The above studies provided the necessary parametric data to enable us to conduct a series of three experiments[57] designed to study the immunomodulatory effects of the conditioned aversive stimulus itself. To control for different factors ancillary to the conditioning process (e.g., nonassociative shock effects and handling of the subjects), our first experiment employed five groups of rats. Two groups were given two consecutive days of conditioning during which there were ten daily presentations on a 4-min VT schedule of either a 15-s light or a 15-s clicker stimulus that coterminated with a 5-s, 1.6-mA foot shock. Then, after a 6-d recovery period in their home cages, both groups were given a single test session involving 10 presentations, on a 4-min VT schedule, of just the light stimulus, which was a conditioned aversive stimulus for group A and a novel stimulus for group B. Because a novel stimulus can elicit fear and possibly generate stress, two other groups were given differential conditioning to light and a clicker in order to familiarize the subjects with both stimuli. On each of their two conditioning days, one group received ten presentations of light paired with the shock, as above, and ten presentations of stimulus a clicker unpaired with the shock. The other group received just the reverse. On the test day, following the 6-d recovery period, both groups also received 10 presentations, on a 4-min VT schedule, of just the light stimulus, which was a conditioned stimulus for the light/shock conditioned group and a nonfunctional, familiar stimulus for the other group. To control for any stressful effects stemming from handling and transportation of the subjects, a fifth group was given the same treatment as the other four groups, except that its exposure to the apparatus during the conditioning and test sessions did not include presentations of light, clicker, or the shock.

Immediately after the test session, the subjects were sacrificed and their spleens removed for mitogen-stimulation assays involving both Con-A and PHA. Those assays showed that there was a comparable pronounced suppression of the reactivity of splenic lymphocytes for the light-conditioned groups relative to the other three groups, which did not differ. Hence, the findings indicated that the observed immunosuppression was due specifically to the acquired properties of the conditioned stimulus and not to the shock experience, per se, nor to handling, differential exposure to a light, or a clicker.

To further substantiate our findings, we conducted two other experiments[57] evaluating whether the suppression of lymphocyte reactivity induced by the conditioned stimulus would be attenuated by operations known to degrade that stimulus as a signal for electric shock, viz., extinction and preexposure. In one experiment, two experimental groups were given the same conditioning, recovery, and test treatments as the light-conditioned group above,

except that conditioning to light was limited to one session and six additional days intervened between conditioning and the recovery phase. On those added days, one group was given extinction training, involving 10 daily presentations of light without the shock on a 4-min VT schedule. The other group was left undisturbed in the home cages. Two control groups received exactly the same treatments as the experiment groups, except that on the test day, the controls were exposed to the conditioning chambers without any presentations of light. (Our use of both controls was to assess whether extinction training resulted in a change in the baseline mitogenic response.) The second experiment duplicated the first, with the exception that the 6 added days occurred prior to, rather than after, the conditioning session and involved exposure to the conditioning chambers with or without 10 daily presentations of light. Such preexposure to a conditioned stimulus is known to produce "latent inhibition" and to retard subsequent conditioning of that stimulus.[58] As before, all subjects were sacrificed immediately after the test session and their spleens removed for mitogen-stimulation assays involving both Con-A and PHA. Those assays showed that although test presentations of a conditioned aversive stimulus induced a pronounced suppression of lymphocyte reactivity, that suppression was significantly attenuated for the experimental subjects that had received preexposure or extinction training. Nevertheless, the lymphocyte reactivity for those subjects was still reliably suppressed relative to the baseline performance of the controls, which did not differ. That outcome speaks to the robustness of our conditioning effects because it indicates that psychological stress in the form of conditioned fear can still suppress immune activity in spite of manipulations specifically designed to attenuate that fear.

3. Stress and Experimentally Induced Autoimmune Disease

The literature on stress-induced alterations of immune function has indicated a paucity of studies examining *in vivo* responses or a disease end point. Thus, we have developed research utilizing an animal model of autoimmunity to examine the interrelatedness of stress-induced immunomodulation and disease. The model is adjuvant-induced arthritis (AIA), an animal model of rheumatoid arthritis. The procedure for AIA involves injecting 0.2 ml of incomplete Freund's adjuvant containing 2.0 mg of *Mycobacterium tuberculosis*, subcutaneously at the base of the tail in male Lewis rats. In approximately 14 to 16 d, 50 to 60% of normal rats begin to develop arthritis in one or more paws. The severity of the disease can then be scored, based on swelling, redness, and deformity of the ankles. A 14-point scale developed by Amkraut and colleagues[59] can be used to determine the severity of the disease. A score of 14 indicates very severe disease in all 4 paws and zero (0) equals no change. The ratings are performed by an experimenter without knowledge of the group treatment. The investigation of this disease is particularly relevant to our investigation of stressor-induced alteration of peripheral blood lymphocytes, as the disease is induced by circulating blood lymphocytes.

The results of our preliminary experiments have indicated that 16 presentations of electric shock 24 h before the administration of the adjuvant decreases the number of subjects developing signs of arthritis from 56% for the nonstressed subject to 25%. The shocks were delivered on a 4-min VT schedule with each shock (5 s, 1.6 mA) being preceded by a 15-s clicker signal. Furthermore, a Student *t* test showed that out of those subjects that developed disease, there was a decrease in the severity of arthritis for the shocked subjects (3.4 on the 14-point scale) compared with the nonshocked subjects (6.1).

In contrast, rats that receive presentations of shock at the time of disease onset (day 14 or 16) showed an exacerbation of arthritis. The exacerbation was exhibited as an increase in severity of the disease for the shocked subjects (10.43 on the 14-point scale) compared with the nonshocked subjects (6.1). These results indicate that the severity and incidence of disease can be modified by presentations of a physical stressor.

One of the paradoxical findings in the human literature is that a stressor decreases

immunocompetence, but has been reported to increase the development of autoimmune disease. Our research provides some insight into that paradox because it demonstrates that the temporal relationship of the stressor, the antigen exposure, and the disease onset are important to the development of the disease and its symptoms.

B. EMOTIONS AND IMMUNITY

Studies with human subjects have suggested that the psychological aspects of a stressful experience can profoundly affect immune function. For example, several investigators have reported an association between spouse bereavement and decreased lymphocyte function.[60] Psychiatric patients reporting a high degree of loneliness and/or a high frequency of stressful life events generally show decreased immunocompetence.[61] Reduced lymphocyte responsiveness also has been reported for patients hospitalized for major depressive disorders.[62] However, the results of these studies are limited in that their assessments were performed solely with blood lymphocytes. Studies of different lymphoid compartments in animals indicate that immune alterations that are induced by stress may differ between the blood, spleen, and lymph nodes.[52]

It has been known that marital happiness can influence health. Individuals who are unhappily married have more health-related problems than those who are happily married or divorced.[63,64] A study of immune function in separated or divorced women and married controls was recently reported.[65] The participants with poor marital quality were found to have significantly elevated antibody titers to Epstein Barr virus (EBV) than the married controls. This may indicate a decrease of cellular immunity with reactivation of a latent virus. In accord with this hypothesis the response of lymphocytes to nonspecific mitogenic stimulation was higher in married individuals. However, the percentage of CD4, CD8, and NK lymphocytes did not differ between the two groups. Whether the immunological changes are reflected in health differences has not yet been determined.

An ongoing comprehensive study utilizing medical students is being performed at Ohio State University by Dr. Ronald Glaser et al.[66] Students have a variety of immunological and psychological tests performed after summer vacation. They are then retested at the time of comprehensive examinations, which take place during the first and second years of medical school. It has been found that academic stress results in an elevation of antibody titers to latent viruses such as herpes simplex virus, cytomegalovirus, and EBV. However, antibody to polio virus does not become elevated, as polio is not a latent virus, and, therefore, this does not represent a generalized humoral activation. Thus, the serological study suggests that virus activation occurs in association with stress. There are also decreases in the percentage of CD4 lymphocytes and NK cells and a functional decrease in NK cell activity.[67-70]

Analysis of the psychological testing shed interesting information in interpretation of the viral antibody titers. Students who were found to have higher loneliness scores on a psychological test[67] had higher antibody titers. Students with low loneliness scores had lower antibody titers than those with higher scores. Thus, this suggests an interaction between loneliness and the inability to maintain virus latency in students when subjected to stress. Stress in susceptible individuals may downregulate the ability of the cellular immune system to suppress latent herpes viruses with a resultant reactivation of the infection. It is important also to consider that factors related to the time of weaning and rearing conditions can have long-term effects on immune system function.[71]

1. Studies in Depression

In recent years, studies of immunologic function in depressed patients have been more numerous than such studies in other psychiatric disorders. This is because of reports indicating a decrease in the quality of health in individuals undergoing depression. In particular, the

loss of a spouse is associated with an increase in mortality of the surviving individual.[72-76] Loss of a child may be associated with an increased mortality only in widowed or divorced surviving parents and this observation is statistically significant for a mother.[77] Loss of an adult son is not associated with increased short- or long-term mortality in married parents.[77] Thus, companionship may participate in determining susceptibility to disease during bereavement.

For the purposes of this review we will limit our discussion to studies of patients who clearly meet criteria for the diagnosis of depression as defined by the Research Diagnostic Criteria or the DSM III.[78] Studies of subjects under stress or experiencing distress but who do not have a diagnosable depression are not included in our review.

A number of studies in depression have examined the response of lymphocytes to mitogens. Among them are those of Kronfol et al.[79] and Schleifer et al.[62] Both groups reported that Con-A and PHA responses were reduced in severely ill, depressed inpatients. Kronfol et al.[80] also reported that the PHA response in depressed patients was lower during acute episodes than during remissions. Calabrese et al.[81] also studied depressed inpatients and also reported that they had reduced PHA and Con-A responses. Schleifer et al.,[82] in a further study, reported that mildly depressed outpatients had normal PHA response unlike the more severely ill inpatients previously studied by them.

Studies of lymphocyte subsets in depressed patients also have been carried out, but the results have not been consistent. Schleifer et al.[62] reported finding lowered T and B cells. Kronfol et al.[83] found that only patients with hypercortisolemia had lowered lymphocyte and neutrophil counts. Kronfol and House[84] also have reported decreased NK cell activity in depressed patients when compared with controls.

Lowey et al.[85] studied PHA response in depressives in relation to high cortisol production and nonsuppression in response to dexamethasone. They found that the administration of dexamethasone reduced PHA response (as expected) only in depressed patients in whom it also suppressed cortisol blood concentrations. Thus, lymphocytes from some depressed patients appear to be more resistant (subsensitive?) to the physiological effects of corticosteroids.

A comprehensive study of the effect of depression on immune function has been reviewed recently by Irwin.[86] In women whose husbands had died recently or were dying from lung cancer, NK activity was significantly reduced in comparison with women whose husbands were healthy. There were no alterations of T lymphocyte populations. A correlation between reduced NK activity and depressed mood and insomnia was found. Also, women who had severe depressive symptoms had significantly more CD8 positive lymphocytes than women with more moderate depressive symptoms.

Whether any of these immunological changes are pathogenetically related to the symptoms of depression or simply nonspecific results of the disorder remain matters of speculation. On the whole, reported findings tend to be similar to those associated with any psychological stressor. Thus, at present, the changes that have been reported in depressed patients suggest that these are the nonspecific effects of psychological distress. It should be added, however, that extensive, detailed, and longitudinal studies of immune function in depression have not yet been published.

C. HOUSING AND IMMUNITY

Immunologists use animals extensively in their research. However, conditions, such as noise in the animal room, when cages were last cleaned, the number of animals in a cage, odors, the sound of other animals in pain, the outside temperature when the animals were shipped, and how they were shipped, are usually not addressed when data are analyzed. We often question the range of results when studying inbred animals and may wonder just how inbred the animals are. Perhaps more attention should be directed to stress factors that may alter immune function.

There have been a number of studies conducted using animal subjects and various housing conditions as an environmental factor to influence disease susceptibility (i.e., malignancy and infection). Although we do not know if it is correct to equate housing with stress, the number of animals housed per cage is an environmental factor that can influence the function of the immune system and is therefore of concern to immunologists. This model of brain-immune system interaction, which is more analogous to everyday human interaction than are many of the laboratory models of stress, provides an interesting opportunity to evaluate modulation of immune system function by the CNS. As will be indicated, change in housing conditions may have a significant effect on immune function.

1. Malignancy and Housing

Many early studies, in which different numbers of animals were housed per cage, noted differences with respect to tumor growth. For example, in 1942 Andervont[87] housed female mice either alone or with eight animals in a cage. The strain of mouse studied is prone to the spontaneous development of mammary tumors. The animals that were housed 1 per cage spontaneously developed mammary tumors at a mean age of 9.6 months, while those housed 8 per cage developed mammary tumors at a mean age of 11.9 months. In addition, 98% of the individually housed animals developed mammary tumors, whereas only 80% of the group-housed animals developed tumors.

Transplanted tumors have also been found to differ in their growth in differentially housed mice. It has been found that both male and female mice of the C57BL/6 inbred strain, when raised in isolation after being weaned, have slower growth of transplanted tumors than animals that are housed in groups of 10.[88]

Studies of differential tumor growth in relationship to different housing conditions may provide an indirect indication of altered immune system function. It is known that tumors arise more frequently in individuals who have suppression of their immune system (e.g., following a tissue transplant), in comparison with those who have a normal functioning immune system. Indeed, one of the major efforts to develop an effective means of inhibiting the growth of malignant tissue is through activation of the immune system. Thus, these early studies regarding tumor growth are significant in that they provided an indirect indication that housing conditions can modify the immune system.

2. Infection and Housing

A number of studies have been conducted on the effects of housing on resistance to infectious agents. One report concerning the effect of housing on susceptibility to infection was on the reactivation of rabies virus in guinea pigs [89] An individually housed guinea pig that had survived for more than 8 months after an initial injection of rabies virus developed clinical signs of infection and died of rabies after being subjected to crowding in a cage with 10 other guinea pigs.

A more comprehensive study of infectivity rates in differentially housed animals was conducted in male and female mice of the CD-1 strain.[90] In this study, the mice were housed individually or in groups of five per cage. Mice were infected with the protozoan organism *Plasmodium berghei* (an agent that causes malaria) and the length of survival for each animal was determined. Both male and female group-housed mice exhibited less resistance to malaria infection than did the individually housed animals. Furthermore, the survival rate was greater in females than males, regardless of housing conditions.

In studies with another common infectious agent *Mycobacterium tuberculosis*,[91] the causative agent of tuberculosis, male mice of the B albino C strain that were crowded (less than 3 $in.^2$ of floor space per animal) and then placed in individual cages (9 $in.^2$ of floor space). These mice showed more resistance to the *M. tuberculosis* infection than did mice that were first housed individually and then switched to crowded conditions. Interestingly,

male and female mice reacted differently to the experimental treatment. Males showed an increased susceptibility to infection in comparison with similarly housed females and crowding increased the resistance of females to *Mycobacterium*. This suggests that males and females differ in their immunological response to environmental conditions.

Caution is needed with respect to generalizing about findings regarding the influence of environmental factors on susceptibility to infection. It is possible that hormonal factors that regulate the immune system may also regulate the ability of infectious agents to multiply or survive attack by the immune system. Possibly some infectious agents may survive better when the temperature of the host is higher and animals housed in a group may have higher temperatures than animals individually housed. Thus, a variety of reasons can be given to explain the discrepant findings.

3. Immunization and Housing

A more refined approach to the study of housing effects on immune responses is the measurement of antibody levels generated in response to immunization with a protein. In one study, male inbred mice were weaned at 20 d of age and then housed either individually or in groups of 5 mice per cage, continuously or for 4 h/d.[92] After 5 d, all mice were injected with bovine serum and the antibody response to the bovine serum determined. Mean levels of antibodies to bovine serum were found to be highest among the mice kept isolated throughout the experiment. Significant differences were not noted between the mice that had been grouped together continuously and those that had been together only 4 h/d. While the kinetics of the antibody responses were identical in all groups tested, the significant difference was in the magnitude of the response. Interestingly, in the above experiments it was noted that dominance or submissiveness of the mice influenced antibody levels, with the dominant animal having the highest level.

Additional experiments that assessed the antibody response to bovine serum in mice that were either isolated, grouped, or first isolated then grouped, and finally reisolated confirm the above findings.[93] The experiment showed that mice in continuous isolation had significantly higher antibody levels than mice in either of the two other groups. Mice that were initially isolated, then grouped, and finally reisolated had the lowest antibody levels. Thus, the greater the number of environmental changes, the greater the reduction in antibody production. It is important that the effect of changing environments, as well as the actual environment, be evaluated for the effect on immune function.

Decreased antibody production by a species other than mice, male rats, living in grouped housing conditions has been reported[89] in support of the earlier findings in mice. The rats were housed 2 per cage or 5 to 6 per cage 1 week prior to immunization and throughout the experiment. The rats were immunized with Salmonella and both initial and antibody responses following reimmunization were measured. Significant suppression of the initial antibody response to Salmonella was observed in the animals housed 5 to 6 per cage. Further, the group-housed rats evidenced near complete absence of a reimmunization response. Thus, although immunologists tend not to consider housing conditions when using experimental animals in their research, this disregarded parameter may influence their results.

4. Detailed Evaluation of How Housing Affects the Immune System

A number of studies have been conducted in our laboratory examining immunologic changes that occur when different numbers of animals are housed per cage. Briefly, those experiments demonstrate the following:

1. Not every strain of mouse shows an alteration of immune reactivity when different numbers of mice are housed per cage.
2. Some strains only show an effect in male mice, whereas others show the effect in both sexes.

3. The effect is transient and may be related more to the change in housing conditions than the actual number of animals per cage.
4. The immune alterations primarily occur at the level of the T helper cell, specifically with respect to the production of a soluble product of T-helper cells termed IL-2.

We have conducted a number of experiments that form a basis for understanding the immunologic alteration associated with housing conditions. Initially, we studied the C3H/HeJ inbred strain of male mice. We obtained the mice from a commercial supplier when they were approximately 6 weeks of age. Upon receipt, they were placed into cages with either five or one animal(s) per cage. The cages were 6 in. wide and 12 in. long.

a. Effect of Housing on T and B Lymphoctyes

Our first study involved the injection of the animals with sheep red blood cells. This antigen was selected because antibody production to sheep erythrocytes requires a T-helper lymphocyte to interact with the antibody producing B lymphocyte before antibody to the sheep red blood cells will be produced. Thus, by using sheep red blood cells as an antigen, we were able to test the functional activity of T-helper lymphocytes and B lymphocytes. Our data indicated that the number of B lymphocytes producing antibody to sheep red blood cells was approximately 50% lower when the C3H/HeJ male mice were housed 5 per cage in comparison with those housed 1 per cage.

We then sought to determine whether the T-helper lymphocyte, B lymphocyte, or both lymphocytes were functionally altered by the housing conditions. To do this we immunized the mice with an antigen that does not require a T-helper lymphocyte to be involved with the antibody production pathway. The antigen that was used was a carbohydrate that directly interacts with B lymphocytes. When the mice were injected with the carbohydrate antigen, equal numbers of antibody-producing lymphocytes were obtained from animals housed either one or five per cage. This indicated that B lymphocyte functional activity was not directly altered by the differential housing conditions. However, when a T-helper lymphocyte is required to facilitate antibody production, less antibody occurs because of a possible altered function of the T-helper lymphocyte.

To further investigate the function of T-helper lymphocytes, two additional experiments were conducted. The first involved stimulation of the T lymphocytes with nonspecific mitogenic agents. The rate of mitotic division induced in the T lymphocytes was significantly greater in the lymphocytes from individually housed animals in comparison with group-housed animals. The second assay involved quantitation of the amount of IL-2 produced by the T-helper lymphocytes from group- and individually housed animals. We found significantly more IL-2 produced by the T-helper lymphocytes from individually housed mice in comparison with what was produced by the group-housed animals. The difference in IL-2 production would account for less antibody production and the lesser response to mitogenic stimulation that we found.

Finally, we determined whether there were differences in the production of IL-1, a product of macrophages, between the group- and individually housed mice. We found IL-1 production to be in group and higher than housed mice.

We have not yet determined why IL-2 production differs in individually and group-housed male C3H/HeJ mice. We do know that it is not because of hormones produced by the adrenal gland. If we remove the adrenal gland from male C3H/HeJ mice and place the animals into the differential housing conditions, the immunologic differences between mice housed five or one per cage persists. In addition, the concentration of corticosterone is the same in C3H/HeJ male mice when housed five or one per cage. Thus, if one equates stress with an elevation of corticosterone in the serum, the observation of an altered immune function with differential housing would not be considered stress. However, other hormones

or nervous system pathways may become altered based on housing. As we understand these pathways better, we may have to redefine the chemical changes that we consider to be synonymous with stress.

b. Effect of Housing on Male and Female Mice

Our next series of experiments involved studying female C3H/HeJ mice. When the female animals were housed five or one per cage, there were no differences in any of the immunological parameters between the two groups. Thus, there is an interaction between sex and the housing conditions in C3H/HeJ mice.

A number of differences are known to exist when the immune systems of male and female animals are compared. Most striking are those that exist in an inbred strain of mouse known as the NZB/NZW. This strain of mouse spontaneously develops a disease that is commonly used as a model for systemic lupus erythematosus in humans. The female mouse develops the disease earlier and has a more severe disease than the male. When the male animal is castrated, it develops the disease as if it were a female. Giving female NZB/NZW mice male hormone converts them to the male course of disease.

Similarly, sex-segregated group housing increased amyloidosis and decreased survival time in male but not in several strains of female mice of several strains.[95] In another study, group housing and a complex environment resulted in thymus involution in CBA male mice, but not in female mice, and in a greater depletion of adrenal cholesterol and ascorbic acid in males.[96]

Sex differences in mortality have been well documented for a wide range of vertebrate and invertebrate species,[97] including humans.[98,99] For example, the lifespan of females was reported to exceed that of males for 12 or 17 strains of inbred and outbred mice.[100] Of significance is the sex difference in susceptibility to infection and disease,[101] which may reflect, at least in part, differential responsiveness of the immune system. While numerous exceptions exist according to species, age, and specified disorder, a clear general trend is for greater male vulnerability.[102,103]

However, it is important to reiterate our caution against generalization. Recently, we identified a strain of mice that has increased immunologic reactivity when individually housed in comparison with being housed five per cage. The CD-1 strain showed a similar increase of immunologic reactivity in individually housed male and female animals. Thus, the CD-1 females differed from the C3H/HeJ females.

Preliminary experiments with castration of male C3H/HeJ mice suggests that the enhanced immunologic reactivity in individually housed animals can be eliminated. However, ovariectomy of female C3H/HeJ animals has not resulted in enhanced immunologic responsiveness in the individually housed mice.

c. Response to an Infectious Agent

In addition to the *in vitro* assays, *in vivo* studies of resistance to infection with *Candida albicans* were also performed. Individually or group-housed animals were inoculated intravenously with *C. albicans* and the number of organisms that had to be injected to infect 50% of the animals determined. Individually housed animals were significantly more resistant to infection, requiring approximately 2.5 times more organisms to infect 50% of the male C3H/HeJ mice.

The above studies were done in the following manner. Animals were obtained from the Jackson Laboratory and, upon receipt, placed into cages with either five or one mice per cage. Approximately 10 to 14 d after the animals had been placed into the differential housing conditions, the differences in the immune system, as well as resistance to *C. albicans*, were found to be significantly greater in the individually housed mice.

d. Differential Housing Produces as Transient Alteration of Immune Function

By 3 weeks after being placed into the differential housing conditions, the immune

reactivity (nonspecific mitogen reactions and number of spleen lymphocytes producing antibody to sheep erythrocytes) and resistance to *C. albicans* were similar, regardless of the number of animals housed per cage. Thus, the immunologic difference is a transient difference and seems to be related to a change in the environment, as well as to the number of mice housed per cage.

e. Genetics and Corticosterone

The immune system is regulated by a series of genes that are located in a region called the major histocompatibility complex (MHC). We wanted to determine whether the MHC was involved in the altered immune function with differential housing. Within the MHC there are two genetic loci of primary importance in the mouse. These are termed H2D and H2K. Strains of animals were selected for study that shared the H2D and H2K loci with the C3H/HeJ animals that shared either or both of these loci with C3H/HeJ. One strain, C3H.SW/SNJ (which shares neither the H2D or H2K loci with C3H/HeJ), did have increased resistance to *C. albicans* and significantly more lymphocytes producing antibody to sheep erythrocytes in the spleens of the individually housed mice. Also, the C3H.SW/SNJ male mice did not have a difference in their corticosterone levels based on housing. Thus, in this respect they were identical to the C3H/HeJ mice. This allows us to conclude that corticosterone levels are not primarily involved in the differential immune response and that the major histocompatibility locus is not involved in the immunologic differences based on housing.

Another preliminary experiment was performed wherein C3H/HeJ male mice were infected with a given sublethal dose of *C. albicans* and 10 d later the number of organisms per kidney were counted. Approximately half of the mice had little or no infections in the kidneys, while the other half had heavily infected kidneys. A group with an intermediate degree of infection was virtually nonexistent. Thus, it appears as if there are two immunologically reactive groups with respect to resistance to *C. albicans*. This may reflect genetic variability for resistance to an infectious agent or the difference between dominant and submissive animals with differing immunologic reactivities. These studies indicate that even within a defined housing condition with inbred mice, variable immune reactivity exists due to factors that have yet to be determined.

V. PSYCHOLOGICAL AND BEHAVIORAL FACTORS IN IMMUNE-MEDIATED DISEASE

A. WHAT DOES NORMAL MEAN IN IMMUNOLOGY AND WHAT IS ITS SIGNIFICANCE TO PSYCHONEUROIMMUNOLOGY?

Stressors can produce alterations of *in vitro* immunologic parameters. However, relating altered *in vitro* findings with clinical disease susceptibility must be done with caution. An example that illustrates the reason for caution in the interpretation of immunological laboratory data is provided by examining the clinical significance of altered immunoglobulin (IgG) levels in humans. The normal concentration of IgG is approximately 1200 mg/dl. Two SD below the normal range is approximately 700 mg/dl. Thus, an individual who had 400 mg/dl of IgG would appear to be severely IgG deficient (in a statistical way) and at an increased risk of developing pyogenic bacterial infections. However, approximately 150 mg/dl of IgG is sufficient to provide adequate resistance against the development of pyogenic infections. Thus, there is a large difference between the physiological normal value and a statistical normal value. Alterations of other immunological values outside of the normal range may or may not have physiological significance. Certainly, a stressor can cause an alteration of some parameters of immune function. Whether these immunological alterations are responsible for increased disease susceptibility cannot be implied simply because a value is less than 2 SD below the mean.

The functional activity of the cellular components of the immune system can be studied in the laboratory. However, there are no clear indications as to the amount of functional activity that is required to prevent problems with infectious disease. Certainly, total suppression of the functional activity of T lymphocytes would be associated with increased disease susceptibility. Yet, the functional activity, even if it is below the normal range, may be adequate for resistance to those diseases that arise with a complete absence of the particular activity.

The clinical consequences of fluctuations in the functioning of the immune system need further study. Covariation of disease activity in conjunction with alterations in stress-induced function of different immune components will be necessary in order to relate stressors to disease exacerbation. The primary diseases associated with decreased immune function are infections, malignancy, and possibly autoimmune diseases. These are the disease categories that should be focused upon.

It is not known whether multiple immunological alterations act additively or synergistically to increase the susceptibility to infectious diseases, malignancy, or autoimmunity. Thus, if a stressor alters several immune functions, even though each by itself may not increase disease susceptibility, having several alterations may produce marked effects on the quality of health. Obviously, there are questions of basic immunology that have not yet been answered. However, as a stressor can alter immune system function, it is entirely appropriate to investigate in both animal and human systems how stress alters immune function and what immunological functions are altered. The question of the types of stressor, the acuteness or chronicity of the stressor, and the ability to cope with stress are all factors that must be considered.

It is important to emphasize that this is a newly emerging field that is becoming increasingly involved with providing information that will help in understanding how one can maintain the quality of their health. If psychoneuroimmunology can show that suppressed immune reactivity can occur by the influence of the CNS on the immune system, and that the CNS is also capable of augmenting the function of the immune system, a significant potential role for psychoneuroimmunology in understanding the pathogenesis of diseases will be achieved. Not only may it be possible to better maintain the quality of one's health, particularly as one ages, but the possibility of altering the course of diseases such as malignancy and autoimmunity may also be achieved.

The following will review evidence indicating that the immune system is susceptible to the influence of the CNS, that the immune system can influence the functioning of the CNS, and possible mechanisms for such influences. We also suggest areas of needed investigation that are important for the clarification and clinical application of many basic science observations.

B. STRESS AND AUTOIMMUNITY

There are a variety of diseases that are believed to have an immunological component to their etiology. There is suggestive evidence, based on our understanding of the pathogenesis of autoimmunity, that demonstrates that a stressor may be associated with disease onset or exacerbation. Retrospective evidence is available for several autoimmune diseases. For example, the onset of insulin-dependent diabetes is frequently associated with a stressor. In children, death, divorce, or separation of the child's parent correlates with the onset of disease.[104] Similarly, the onset of Crohn's disease has been associated with stressful life events in patients.[105] Examples of the stressors associated with the onset of Crohn's disease include bereavement, pregnancy, marriage or divorce, and relocation. In Crohn's disease, a stressor is related to both the onset of disease and relapse.

Rheumatoid arthritis has also been reported to develop and be of a greater clinical severity in patients undergoing a stressor.[106] In particular, female patients are found to be

more nervous, tense, and worried than their healthy female siblings. In patients with rheumatoid arthritis who experience emotional decompensation, a more rapidly progressing disease with more incapacitation and a poor response to medical therapy will frequently occur. Thus, in rheumatoid arthritis the course of disease may be related to the integrity of the patient's psychological defenses.

Uveitis undergoes recurrence in individuals who have recently lost a spouse or undergo other stressors such as divorce, business loss, or the failure of not passing an examination.[107] Uveitis will respond therapeutically to immunosuppressive drugs. Thus, Uveitis provides an example of another disease in which immune activation may participate in the disease pathogenesis.

One of the diseases that has been best studied with respect to the association between disease onset and stress is Graves' disease. Indeed, Volpe[108] has stated that the conclusion is inescapable and that in some patients there is a cause-and-effect relationship between stress and the development of hyperthyroidism. Stressors associated with the onset of Graves' disease include rigorous dieting, bereavement, marital discord, or infection.

Autoimmune disease develops when the immune system becomes sensitized to autologous tissue. This may occur secondarily to an infection that may alter the structure of self antigens, so the immune system believes them to be of a foreign nature. If a stressor can suppress the function of the immune system with a resultant increased likelihood for infection, there may be an increased susceptibility to the development of autoimmune disease associated with infection and antigenic alteration. Indeed, there is substantial information that indicates that viral infections are associated with the onset of autoimmune diseases such as Hashimoto's thyroiditis and insulin-dependent diabetes.[109]

Alternatively, an immune response directed against the infectious agent, in an attempt to eliminate the infectious agent, may produce secondary damage to the tissue that is infected. An example of this is a patient who is infected with the hepatitis B virus. The immune response directed to the virus that is present on the surface of hepatocytes damages the hepatocytes. Once the virus is eliminated, the immune response is over and, in the case of liver, regeneration and normal function occur. However, if the immune system does not function properly and does not eliminate the virus, a chronic immune reaction takes place with persistent damage to hepatocytes. A patient with this occurrence is labeled as having chronic active hepatitis. Thus, an interaction may exist between chronic disease and chronic stress in which a chronic disease functions as a stressor and the disease course may be influenced by immune changes produced by chronic stress.

If a virus is trophic for the insulin-producing cells of the pancreas (beta islet cells), an immune response to the virus will damage the beta cells. Once the virus is eliminated, the immune response will cease and the individual will be left with a decreased number of beta islet cells, as the cells do not regenerate. However, the individual will not become diabetic as approximately 20% of the beta islet cell numbers can maintain normal glucose levels. However, if the individual has an immune system that does not eliminate the virus, a chronic immune response will occur and eventually all of the beta islet cells will be destroyed. Whether stressors play a role in this mechanism of tissue destruction with resultant disease is an area of needed investigation. There are several reports that indicate an increased risk of diabetes in children who are experiencing stress.[110] The combination of a virus that is trophic for any endocrine tissue in combination with an impaired immune system may lead to immunological damage (autoimmune disease) of that tissue.

Another pathway by which an autoimmune disease may develop is associated with loss of regulation of the immune system. This theory assumes that lymphocyte populations capable of exerting an autoimmune reaction are normally present in all individuals, but that the cells are functionally suppressed by the presence of the T-lymphocyte suppressor population. If the T-lymphocyte suppressor population becomes functionally depressed, autoimmune re-

actions may evolve. Whether the T-suppressor lymphocytes themselves become suppressed or whether an inducer cell of the suppressor cell becomes suppressed has not been determined. Indeed, both mechanisms may occur. Thus, if a stressor is capable of depressing the functional activity of a cell population that normally downregulates an autoimmune reaction, an autoimmune disease may result.

The reason why the autoimmune disease involves a specific tissue (organ specific autoimmunity) suggests that altered immune function in conjunction with an event occurring in the organ that is diseased may be necessary. Thus, two events may be needed for an autoimmune reaction to develop. If a stressor increases susceptibility to a viral infection (capable of altering the antigens of the organ or stimulating an immune response to the infectious agent and secondarily damaging tissue) and the stressor downregulates the immune response, the single event of experiencing a stressor may provide the mechanism for both components needed to develop an autoimmune disease.

It is unlikely that a stressor is capable of altering the antigenicity of the tissue. Therefore, we must assume that finding an association between a stressor and the onset of exacerbation of autoimmune disease is due to the influence of the stressor on immune system function.

We associate an autoimmune disease with an active immunologic response. Thus, if a stressor suppresses the immune response there may be stress-induced selective suppression of the regulatory lymphocyte population (T-suppressor cells) in some individuals with resultant increased activity of other parts of the immune system. This is not to imply that a stressor will always alter the T-suppressor cells in all individuals undergoing stress. There may be a variety of hormones, some of which alter the functional activity of T-suppressor lymphocytes, while others may alter the activity of the T-helper lymphocytes. Whether the different pathways are related to genetic factors, chronicity, or type of stress, or if indeed they exist, are areas of needed research.

C. PSYCHOSOCIAL FACTORS AND INFECTIOUS DISEASE

On exposure to an infectious agent, only a small proportion of people develop clinical disease. Moreover, severity and duration of symptomatology vary widely among those who do become ill. Although other biological systems may contribute, susceptibility to infection is presumed to be primarily influenced by immune function. As discussed earlier, there is burgeoning evidence that the immune system is modulated by psychological factors such as stress. These same factors would be expected to influence susceptibility to infectious disease through immune pathways.

Do stress-induced changes in immune function have implications for either the onset or course of infectious disease? Although stress has been related to immune modulation as assessed by measures of cellular function, persons with high levels of stress and psychological distress do not typically demonstrate levels of immune function outside of normal parameters. There is little evidence on the relation between variations in normal immune function and disease susceptibility, and hence psychoimmunology is left with two questions: Does stress influence immune function, and, if it does, is the nature and magnitude of that influence sufficient cause for alterations in disease susceptibility?

We present evidence on the relation between stress and infectious disease to emphasize the potential importance of CNS involvement in immunomodulation. However, it is important to keep in mind that stress may influence disease pathogenesis through alternative pathways. For example, stress-triggered hormones, such as cortisol or epinephrine may have direct effects on involved tissues, such as increasing mucus secretion and vasodilation.[111] Stress may also influence disease pathogenesis through behavioral changes that occur as adaptations or coping responses. For example, increased smoking under stress could irritate nasal and lung tissues, and failure to adhere to medical regimens under stress could result in a more severe and longer-lasting illness. Moreover, behavioral adaptations such as smoking and

drinking could themselves influence immune function without any direct CNS involvement. Unfortunately, existing studies on the role of stress in infectious disease do not generally provide information to distinguish between these various pathways.

The human literature relating stress to infectious disease is correlational rather than experimental in nature. That is, persons reporting high stress are compared to those reporting relatively lower levels of stress in terms of their risk for developing disease. Although such relations suggest the possibility that stress makes people vulnerable to infectious agents, they may also be attributable to the disease (or premorbid pathology), causing greater stress, or to a third factor, e.g., social class, putting persons at higher risk for both stress and disease. Our review is limited to prospective studies where subsequent disease is predicted from stress levels in *initially healthy* persons. In this way, the interpretation that the disease caused the stress can be eliminated. "Illness behaviors", such as self-reported symptoms and utilization of health services, are used as measures of disease in many studies. Because these measures do not always reflect underlying pathology (infection), we also focus on studies that include a biological verification of disease, for example, virus isolation or cultures screened for bacterial infections. There are only a few studies meeting these criteria and they are limited to work on stress as a risk factor in upper-respiratory infections (URI) and herpes virus infections.

1. Upper-Respiratory Infections (URIs)

Exposure to pathogens responsible for minor respiratory infections is not sufficient cause for the development of subclinical or clinical disease. For example, 30% of a school-age population can harbor group A streptococci without developing symptoms,[112] three quarters of preschool children infected with Mycoplasma pneumoniae remain asymptomatic,[113] and as many as 42% of upper-respiratory tract cultures from well children yield pneumococci [112] In general, the causes of susceptibility to infectious disease are not well understood, but interest in the influence of psychosocial factors, especially psychological stress, has led to some provocative studies.

In an early study, Meyer and Haggerty[114] followed members of 16 families for a 12-month period. Family diaries were used to record stressful life events that disrupted family or personal life. Throat cultures (screened for streptococcal infections) were made every 3 weeks and at times of acute illness. Acute stress was four times more likely to precede than to follow new streptococcal and nonstreptococcal infections and associated symptomatology. In addition, chronic family stress was related to greater numbers of new infections, prolonged carrier states, higher streptococcal illness rates, and elevated antistreptolysin zero responses.

Similar results were reported in a recent study by Graham, Douglas, and Ryan.[115] Measures of life stress were collected from members of 94 families before and during a 6-month period in which diary data on respiratory symptoms were collected daily. Illness episodes were validated by nose and throat cultures. Although high- and low-stress groups were almost identical with respect to demographics and health practices, the high-stress groups experienced both more episodes and more symptom days of respiratory illness.

In a 6-month study,[116] 24 telephone operators had weekly assessment of antibody levels, throat cultures taken, and nose and throat carefully examined for symptoms of infection. Moreover, each subject kept a diary of symptoms and of their important activities during the week. Numbers of both emotionally significant events and of episodes of respiratory illness were small and no definitive results were found. However, the data suggested that events that caused sadness or weeping, periods of sexual excitement, and other events causing pleasurable excitement and arousal precede acute respiratory illness.

Two studies have addressed psychological susceptibility to influenza. In the first,[117] 480 male employees of a military research installation completed a series of questionnaires assessing psychological distress 6 months before the onset of an epidemic of Asiana Influenza.

On the basis of the tests, they were classified as either psychologically vulnerable or nonvulnerable. During a subsequent epidemic period, all persons presenting an influenza-like illness were followed over a 3-week period to evaluate the acute disease. Serum was collected during the acute illness and 3 weeks after diagnosis to verify infection. Risk of reporting illness in the psychologically vulnerable group was about three times higher than in the nonvulnerable men. However, there were no actual differences in infection rates or severity of illness.

In another study, Imboden, Canter, and Cluff[118,119] examined the predictors of *speed of recovery* from influenza in a sample of military employees. They administered psychological questionnaires to 600 persons and then focused on the 26 members of this group who reported to the dispensary with flu during the following winter. All except seven of these cases were confirmed by serology or virus isolation. The 26 persons were classified as recovered if they reported no symptoms 3 to 6 weeks later, and symptomatic if they continued to report symptoms. Symptomatic persons had reported more physical and psychological symptoms, especially symptoms of depression. Although incidence of influenza was verified in this study, there was no biological verification of recovery time. Hence, these results, like those in the previous study, could be attributable to psychological influences on symptom reporting instead of on diseases pathogenesis.

In sum, there is suggestive evidence from these prospective epidemiological studies that stress increases risk for upper-respiratory disease. However, in two studies these effects may be attributable to stress-induced symptom reporting as opposed to stress-induced infection or clinical symptomatology. Two studies similarly suggest that stress lengthens recovery time, but disease duration is not biologically verified in either case.

Another problem in interpreting these studies is that increased incidence of URI under stress may be attributable to stress-induced increases in *exposure* to infectious agents, rather then stress-induced immunosuppression. For example, persons under stress often seek out others, consequently increasing the probability of exposure. Three additional studies addressed this problem by exposing volunteers to specific infectious agents. In the first study,[120] 52 healthy volunteers were given a stressful-life-events interview that resulted in five different stress scores. Subsequently, they were inoculated with two rhinoviruses and followed daily (in isolation) for 1 week. After controlling for initial neutralizing antibodies to the two viruses, total amount of virus shedding was predicted by one of the stress measures, resulting in change in the total level of activity. However, the other four stress scales did not predict virus shedding and none of the stress measures were related to symptom scores.

In the second study, Green et al.[121] examined the effects of self-reported life events and moods on 33 subjects receiving nasal inoculations of an influenza virus (A Victoria) and the drug isoprinosine. The subjects were confined in a motel for 7 d. Life events and mood states were assessed on day 1, and symptoms were rated for day 1 and twice daily for the remainder of the week. On the 2nd day, subjects received nasal inoculation of influenza (A Victoria). Neither stress nor moods were related to antibody production nor viral shedding.

Finally, in a study with a larger sample,[122] 125 volunteers received a "swine" (A/NJ/76) flu vaccine and completed life events and mood scales. Specific neutralizing antibodies were assessed before and 2 weeks after the viral challenge. No relations were found between the psychological measures and postchallenge antibody levels.

Although these three studies are important because of their control over viral exposure, their failure to find consistent effects of stress on susceptibility is difficult to interpret. Individual studies suffer from insufficient sample sizes, the concurrent administration of drugs, lack of information on overall rates of infection in response to the dose of virus administered, and controls for important predictors of susceptibility, such as gender, age, and number of previous infections.[123]

Overall, there is enough evidence supportive of a relation between stress and URIs to

suggest that further work is worthwhile, but not enough to make any definitive conclusions. If anything, this early work indicates the complexity of studying this issue and interdisciplinary sophistication required to design adequate tests of the hypotheses that persons under stress will be more susceptible to infection.

2. Herpes Viruses

A number of studies have addressed the role of psychological factors in infection and recurrence of lesions of the human herpes viruses. Included are studies of herpes simplex type 1 (HSV-1), herpes simplex type 2 (HSV-2), and EBV. HSV-1 is most frequently associated with cold sores, HSV-2 with genital lesions, and EBV with mononucleosis. However, herpes viruses have the ability to produce a range of illnesses, for example, HSV-1 can also produce generalized infections and encephalitis.[124] Herpes viruses differ from most other known viruses in that after exposure they are present all of the time, although often in latent states. Competency of the cellular immune response is thought to be a critical factor in limiting primary herpes virus infections, as well as in subsequent control of latent virus.[125]

3. Epstein-Barr Virus (EBV)

Evidence for the influence of stress on mononucleosis is reported in a 4-year prospective study of a class of 1400 West Point cadets.[126] Cadets who were susceptible or immune to mononucleosis were identified by the presence or absence of antibody to the EBV, and new infections were identified by the appearance of the antibody (seroconversion). Cadets with the combination of high motivation and poor academic performance were more likely to seroconvert, to develop clinical mononucleosis if they seroconverted, and spend more time in the hospital if they developed clinical mononucleosis.

4. Herpes Simplex Type 1 (HSV-1): Oral Herpes

Several anecdotal studies based on highly selected, very small samples suggest that stress plays a role in triggering oral herpes recurrence.[127] However, more rigorous investigations of the relation between stress and recurrence result in tentative but not conclusive support for this hypothesis.

Early support for stress-induced lesions was reported by Katcher et al.[128] These investigators administered psychological tests to 38 young women entering nurse's training and then monitored them for 1 year. The women were asked to report the onset of cold sores that were then verified by oral examination and isolation of HSV-1. Women reporting unhappy moods were found to have elevated episodes of herpes during the following year.

An attempt at replication by this same research group was unsuccessful.[129] In this case, the sample consisted of 43 young student nurses who had a positive antibody titer to HSV-1. The subjects filled out a mood scale daily for 3 weeks and were checked daily for herpes sores on lips and herpes virus in mouth secretions. A daily calendar was kept by each woman containing a notation of cold sores, other illnesses, and concommitment life events. Mood scores prior (and subsequent) to illness episodes were unrelated to the onset of herpes, URI, or aphthous ulcers. Moreover, neither mean mood scores (collapsing over all days of the study) nor variance in mood scores were related to illness incidence.

In another prospective study, Friedmann et al.[130] followed 149 student nurses for 3 years. At the onset of the study, HSV-1 antibody titers were assessed as well as history of primary and recurrent herpes infections. Participants also completed measures of enduring mood trait characteristics. Incidence of herpes recurrence were reported on daily calendar forms. Previous data had indicated that calendar reports of recurrence were consistent with documented lesions. Although the best predictors of recurrence were greater incidence in the past and HSV antibody levels, those reporting more unpleasant moods had more incidence of recurrence.

5. Herpes Simplex Type 2 (HSV-2): Genital Herpes

The literature on psychological influences on HSV-2 is primarily anecdotal and descriptive. Although many patients report an association between stress and recurrences, data supporting this relation are scant and inconclusive.[127] One prospective study does, however, suggest the importance of psychological distress in recurrence.[131] In this study, 58 patients diagnosed (virus isolation) with the first occurrence of genital herpes were administered a measure of psychological distress and then followed for 30 weeks. Those with high levels of psychological symptomatology had a higher rate of verified (by culture) recurrence than those with lower symptomatology.

In sum, there is suggestive evidence that reactivation of latent herpes viruses can be triggered by emotional distress Although stress-induced immunosuppression is often presumed to be responsible for these relations, especially cellular immunity, none of the existing work addresses the role of this biological pathway. The one study of mononucleosis found that persons under stress were more likely to become infected, develop clinical disease, and spend more time in the hospital.

We have been cautious about making strong inferences from existing studies of stress-induced infectious disease. However, this literature looks better when viewed in the context of our ignorance in approaching the problem. This work has been done without the information necessary for guiding the design of studies that provide a fair test of the hypothesis. We know too little about the relation between stress and immunity and virtually nothing about the relation between immunity and disease susceptibility. What are the characteristics of stressors that trigger immune modulation? How long an exposure to stress is needed to influence immunity? How long after stress exposure do immune changes last? Do changes within normal immune parameters have implications for the onset and course of infectious disease? If so, what immune parameters are important and which are not? How long do immune changes need to last before they influence disease pathogenesis? These questions are further complicated by the likelihood that the relation between immune status and disease susceptibility is different for different infectious agents.

In sum, the evidence for stress-induced infectious disease is provocative. However, it is important to remember that relations found in these studies are not necessarily attributable to stress effects on immunity, but may instead be attributable to stress influences on other biological systems or on behavior. Studies that measure viable endocrine-immune pathways, direct effects of stress on other disease relevant biological systems, and stress-induced behavioral changes that put persons under greater risk for disease would be helpful in clarifying responsible mechanisms.

D. STRESS AND MALIGNANCY

It is difficult to speak in general terms of a relationship between the CNS and susceptibility or resistance to malignancy. Cancers involve a variety of tissues, various cells within each tissue, occur in response to different etiologies, occur in individuals of different ages and with different deficiencies to their immune systems, and are detected at different stages of tumor growth and spreading. Some malignancies are considered extremely aggressive, while others have a more indolent course. Thus, it is probably unrealistic to assume that the CNS influence upon the immune system can uniformly participate as a therapeutic modality in patients with malignancy.

An example of the variable efficiency of the immune system in participating in the control of malignant tissue is provided by studying the effect of various biological response modifiers on tumor growth.[132] A variety of substances that may enhance the activity of the immune system have been used therapeutically in patients with cancer. The one consistent theme that has emerged is that patients with malignant melanoma tend to respond better to such immunological enhancement in comparison with patients with other malignancies. It

is important to note that patients with malignant melanoma will frequently undergo spontaneous remission of their disease or regression of tumor nodules may occur at the same time that other nodules are appearing. This suggests that malignant melanoma may be a unique tumor, possibly being of high antigenicity, and therefore capable of stimulating a vigorous immune response. Therapies using IL-2 and lymphocyte-activated killer cells have also suggested that malignant melanoma is more susceptible to immunological influence than other tumors.[133] Obviously, it must also be considered that psychological factors may have a greater influence on some diseases and play a minimal role in others.

In evaluating the influence of the CNS on development or progression of tumors, it is obvious that careful attention must be paid to the type of tumor, the amount of tumor present at the time of study, the use of prior therapies or concurrent therapies, the age of the patient, and the nutritional status of the patient. Without careful attention being directed to the relation between the clinical state of the patient and the function of the immune system, proper interpretation of data regarding the influence of the CNS on tumor growth may not be obtained. For example, comparing the effect of behavioral factors in patients with localized, small, newly diagnosed malignancies with the effective behavioral factors in patients with metastatic disease having undergone high-dose chemotherapeutic therapy is highly inappropriate.

With respect to breast cancer, parameters such as the number of involved lymph nodes, the histopathology of the tumor, the hormone receptor status of the tumor, and whether the patient is menopausal are all factors that may influence the outcome of disease. Also, genetic alterations associated with amplification of the C-*myc* and C-*erb*B-2 proto-oncogenes may be factors that are involved with disease outcome.[134] Thus, in evaluating the effect of behavioral factors on tumor growth and patient survival, it is inadequate to lump all patients together regardless of other factors that influence disease outcome.

If, as has been suggested in a number of studies, patient survival can be influenced by behavioral factors, one must determine the reason for enhanced survival in patients who may meet behavioral criteria, indicating that they will be fighters.[135] If long-term cancer survivors who are fighters are found to have different concentrations of neuropeptides than individuals with greater passivity, it leads to the question of how neuropeptides may participate in resistance to the tumor. Obviously, one first thinks that there is an associated enhancement of immunological function and, indeed, there are studies that indicate that the immune system can function in different ways in individuals who are being stressed in comparison with those not being stressed. Of course, it will be important to determine which of the immunological functions are important in tumor resistance and to carefully determine what psychological parameters influence the immune factors. Alternatively, the neuropeptides, or other hormones that are produced in response to neuropeptides, may bind to the tumor cells and influence tumor growth.[136]

In conjunction with animal studies, there is the suggestion that human malignant disease may be influenced by behavioral factors. Mortality from cancer has been reported to be increased in patients with affective disorders in comparison with the general population.[137] However, as previously indicated, these studies must take into consideration the variety of biological factors that also can influence tumor growth. Whether the behavioral factors influence immune system function or other biological factors is entirely speculative.

VI. ALTERATION OF CNS FUNCTION BY THE IMMUNE SYSTEM

The brain has often been regarded as being protected from immunological surveillance (immunologically privileged). However, several brain disorders are now known to be mediated by specific immunological reactions against antigens found in the CNS. Multiple

Sclerosis, in which the immunological reaction is directed against components in the myelin sheath of nervous tissue, is one example. Acute disseminated encephalomyelitis is another. Therefore, the brain is not completely protected from immunological attack. In fact, for years a laboratory model for antibrain autoimmunity has existed in the form of experimental allergic encephalomyelitis (EAE). EAE is a demyelinating disease that can be produced by the immunization of animals with extracts of whole brain as well as with certain specific brain proteins along with Freund's adjuvant. Thus, it is clear that gross brain dysfunction can result from an immunological reaction against specific brain antigens. Can more subtle dysfunction of the brain, such as disordered thoughts, reasoning, and perceptions, also be mediated through immunological mechanisms? Below we present evidence from studies conducted by us and others that suggest that some of the psychopathological symptoms associated with schizophrenia may be related to an antibrain immunological pathogenesis.

As early as 1937, Lehmman-Facius[138] published the hypothesis that some abnormality of immune function might be pathogenetically involved in the production of symptoms in schizophrenia. Since then, there have been numerous studies reporting that a variety of immunological abnormalities can occur in schizophrenics. These include: elevation of serum IgG levels;[139,140] increased IgG in the CSF;[141] the presence of activated T cells, as determined by morphology in the peripheral blood;[142,143] and functional impairment of T lymphocytes.[144,145] However, these studies have not been guided by a particular etiological or pathogenetical hypothesis, and this has limited the conclusions that can be drawn from them.

Studies that specifically address the autoimmune hypothesis are fewer. Heath et al.[146-148] reported the detection (by immunofluorescence) of a circulating antibody directed against antigens in the area of the septal nuclei. They also reported that serum from schizophrenics, when injected into monkeys, caused specific behavioral and EEG changes that were never produced by sera from normal controls. This work remains controversial because of some failures to replicate the findings as well as methodological concerns. In fact, a partial replication of Heath et al.'s findings was reported by Bergen et al.[149] Other specific findings of antibrain antibody in schizophrenics were by Baron et al.[150] and Pandey et al.[151]

Our own work, reported below, has tried to avoid the methodological shortcomings of the earlier studies, as well as making use of a model derived from our knowledge of the pathogenesis of autoimmune disease in general. In order to do this we have studied the pattern of results on several immunological tests in diseases of known autoimmune etiology, such as insulin dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA). From this we have been able to predict the pattern of findings that would support the hypothesis that autoimmune mechanisms do participate in the pathogenesis of schizophrenia.

The following results are from studies conducted at the University of Pittsburgh over the last 5 years. Schizophrenic patients involved in these studies all meet the Research Diagnostic Criteria for Schizophrenia,[152] as well as DSM III[153] criteria for schizophrenia or schizophreniform disorder.

A. AUTOANTIBODY PRODUCTION AND AUTOIMMUNE DISEASE

There is a strong association between autoimmune disease and the overproduction of autoantibodies in general. It is hypothesized that, in these diseases, there is a failure of normal immune regulation and that the loss of suppression of autoreactive clones of lymphocytes may be crucial in the pathogenesis of disease. In the case of IDDM, screening of all diabetics for islet cell antibody rarely led to positive results. However, when only IDDM patients who had antibodies to other tissues were screened, a high percentage was found to have islet cell antibody. Thus, the production of multiple autoantibodies suggests the propensity to develop an autoimmune disease. Furthermore, if the failure of immune regulation in an individual is generalized, then more than one autoimmune disease may occur simultaneously.

In an initial study,[154] we studied 28 consecutive admissions for schizophrenia to our inpatient unit. We screened for antibodies to parietal cells, mitochondria, smooth muscle thyroglobulin, thyroid microsomes, antinuclear antibodies, and rheumatoid factor.

We found that 13 patients had at least 1 autoantibody and 6 patients had 2 or more. Also, nine patients had a history of an autoimmune disease such as hypothyroidism, hyperthyroidism, and IDDM. As expected, there was a highly significant association between the occurrence of multiple antibodies and autoimmune disease.

Since then we have studied 103 schizophrenic patients further and compared them with 100 roughly age- and sex-matched controls. Schizophrenics showed greater production of autoantibodies and a much higher incidence of autoimmune disease.

B. LYMPHOCYTE SUBSETS

As mentioned, several investigators have reported alterations in percentage and number of lymphocyte subsets in schizophrenia. We have compared 123 schizophrenics with 100 controls on the percentage of lymphocytes, percentage and number of T cells, percentage and number of activated T-helper cells, and the other helper suppressor ratio (H/S). The percentage of lymphocytes was increased in schizophrenics, but only in those on medication. This change is therefore probably related to treatment rather than the disease. Only unmedicated schizophrenics had a reduction in the number and percentage of T cells. Treatment in the case of this variable may normalize T-helper cells. Only the percentage of T-suppressor cells was reduced in all groups of schizophrenics whether they were on medication or not. A decrease in suppressor cells does suggest a possible mechanism whereby autoreactive clones of lymphocytes may become activated. To further investigate why suppressor cells are reduced in schizophrenic patients, we further characterized the T-helper cells into specific subsets that induce the proliferation of other T-helper cells (inducers of help) vs. the subset that induces the proliferation of suppressor cells (inducers of suppression). Schizophrenics had a significant reduction only in the percentage of inducers of suppression as compared with controls.

C. INTERLEUKIN-2 (IL-2) PRODUCTION

IL-2 is one of the lymphokines produced by activated T-helper cells. The normal functioning of all immunoregulatory cells depend upon appropriate IL-2 production. IL-2 production is reduced in several autoimmune diseases such as IDDM,[155,156] SLE,[157,158] and RA.[159] Low concentrations of IL-2 may preferentially stimulate helper T cells, while higher levels are required for T-suppressor cell proliferation. Thus, low IL-2 production could lead to a reduction in suppressor cells.

In a study of IL-2 production in 70 schizophrenics compared with 50 normal controls, we found that schizophrenics produced lower amounts of IL-2 than controls. Furthermore, acutely ill patients had even lower levels of IL-2 production than remitted patients. Of the acutely ill patients, 13 were studied before they received any medications and were found to have the same level of IL-2 production as medicated patients. This rules out the possibility that the decrease in IL-2 production in schizophrenics is due to antipsychotic medication. Preliminary results from ongoing studies also suggest that serum from schizophrenics contains abnormally high levels of solubilized IL-2 receptor.

Other preliminary studies indicate that IL-2 may be increased in the serum of patients with schizophrenia. If this is confirmed, it may indicate that the low IL-2 produced by lymphocytes from patients with schizophrenia is secondary to the lymphocytes having released their IL-2 into serum. Thus, a state of hyperactivation of lymphocytes would be present in schizophrenic patients and this would then correlate with the increase in IL-2 soluble receptor. We are conducting extensive studies to further elaborate on these preliminary findings.

D. CELL-MEDIATED IMMUNITY AGAINST BRAIN ANTIGENS

In IDDM, damage to pancreatic tissue appears to be mediated principally by T lymphocytes.[160] Other *in vitro* evidence of cell-mediated immunity against pancreatic islet cells (lymphocyte transformation and leukocyte immobilization factor) have also been demonstrated in lymphocytes from patients with IDDM.[161,162] If similar autoimmune mechanisms were present in schizophrenia, then sensitization of lymphocytes to antigens derived from brain would be expected. Our earlier work[154] indicates that the strongest immunoreactivity, both humoral and cell-mediated, occurs when antigen is derived from human hippocampus and parahippocampal gyrus.

Brain tissue was obtained from nonpsychiatrically ill individuals who had died in a traffic accident. A cytosol fraction of hippocampus and parahippocampus was prepared, as described in detail elsewhere.[163] A similar saline extract of kidney was also prepared. Isolated peripheral blood lymphocytes were then incubated with the saline extracts of the various brain regions and kidney for 5 d. Mitosis was quantified by the incorporation of tritiated thymidine. A stimulation index (SI) was calculated, based by dividing the counts in cultures containing antigen by the counts in cultures without antigen. Previous experience with this method suggests that SIs above 1.7 are rarely encountered in controls, and therefore this was arbitrarily chosen as the lower cutoff for a positive reaction. A larger proportion of schizophrenic patients had enhanced mitogenic responses that were specific to brain tissue.

E. CIRCULATING ANTIBODY TO BRAIN TISSUE

Reports of the detection of circulating antibody against brain tissue have generated the most excitement as well as the most controversy in the history of immunological studies in schizophrenia. Criticisms have been mostly about the methods employed to detect circulating antibody. In fact, as we have discovered ourselves, the definite demonstration of a specific antigen antibody reaction against brain proteins remains a difficult issue.

Our own earlier studies used an ELISA assay and Western blotting to determine the presence of antibody to a saline extract of brain.[163] The ELISA showed no difference in the proportion of patients and controls showing antibody to antigens from the hippocampus and superior parietal region. The Western blotting did detect binding to certain bands only in patients. However, immunohistochemical studies also showed a high level of nonspecific IgG binding to brain tissue. Our subsequent efforts have therefore been focused on developing methods that allow us to clearly distinguish between specific and nonspecific binding.

It has been shown that nonspecific binding of IgG is more susceptible to inhibition by high salt concentrations. Therefore, we have studied the effect of increasing buffer ionic strength in the ELISA assay for antibody to brain and have found that nonspecific binding is significantly inhibited in 1 *M* salt. To further define the specificity of the antibody reactivity we have also coated the ELISA trays with protein from specific bands obtained from isoelectrically focusing the brain extracts. Only sera from some schizophrenics, but no control, was found to demonstrate specific antibody to a brain antigen with a basic pH.

Taken together, our studies of schizophrenic patients provide strong preliminary evidence that a subgroup of these patients do have many findings similar to those in patients suffering from known autoimmune disorders. The precise mechanism by which these immunological factors participate in the pathogenesis of symptoms remains to be elucidated. In uncovering these mechanisms, we may also learn considerably more about how the immune system and the brain interact and influence each other.

VII. SUMMARY

We believe that any questions regarding whether the CNS can alter immune system functions no longer remain. It can conclusively be stated that the immune system is sus-

ceptible to influences of the CNS. It remains to be determined whether all classes of lymphocytes, NK cells, macrophages, polymorphonuclear leukocytes, and other antigen-processing cells are all susceptible to CNS influences.

We have presented evidence that peripheral blood lymphocytes may not reflect the immunological activity of lymphocytes within lymphatic tissue after being influenced by a stressor. Thus, all types of immunological cells must be evaluated in different organs.

Whether the immune system of young and old animals respond in the same way must also be determined. The sex of the animal needs to be taken into consideration.

What immune responses are important to measure? Do *in vitro* responses reflect the ability of an animal to resist infectious disease or susceptibility to autoimmune and malignant diseases? Certainly, absence of an immune response is detrimental to health. It must be determined whether moderately suppressed immune function in multiple compartments is as detrimental as total absence of an immune response in a single immunological compartment.

The data that we have presented with respect to adjuvant arthritis indicate that an immune response in the peripheral of the animal can be modified by a stressor and influence an immunopathological process. This may indicate that the most important immune compartment to evaluate with respect to altering disease susceptibility is the peripheral blood and that lymphoid tissue may be interesting, but not clinically relevant. The reasons why the peripheral blood and lymphoid tissue differ in their immunological function following exposure to a stressor must be determined.

We have reviewed information indicating that lymphoid tissue is innervated and that such innervation can modify immune function. In addition, hormones released by the CNS may alter immune function. Yet, much of this data are contradictory and whether immune enhancement or suppression occurs is not clearly defined with respect to any experimental manipulation involving denervation or the addition of hormones to *in vitro* cultures. Whether this reflects the age of the experimental animal, the type of immune response being measured, the adequacy of the experimental procedure, background rearing conditions of the animals, the amount of noise in the animal room, the diet of the animals, or the number of animals housed per cage all remain to be determined.

Our purpose has not been to provide a comprehensive review of all of the data relating to the immune system/CNS interaction. Rather, we have attempted to provide enough information so that the reader will be convinced of the existence of this interaction and some of its possible mechanisms. It is hoped that a better understanding of psychoneuroimmunology will assist in enhancing the healthy individual's quality of health. We believe that enormous health benefits and resultant economic benefits may be gained if psychoneuroimmunology can be shown to be capable of enhancing the quality of health by interfering with the development of immunological-mediated disease. We predict that just as stressors may interfere with immune system function, feelings of well-being may promote immune system function.

The science of psychoneuroimmunology is just beginning to be explored. The next decade promises to be increasingly exciting as a better understanding of the CNS/immune system interaction is obtained.

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