Stress-Induced Modulation of the Immune Response

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INTRODUCTION

The suggestion of and the evidence for an integration of the immune system with the central nervous system (CNS) and with the endocrine system has many roots as a result of both human and animal studies. The complex interactions among these three systems have been the subject of many recent studies in the rapidly developing field of "psychoneuroimmunology" or more appropriately "psychoneuroendocrinimmunology" to account for the endocrine system's known contribution in immune function modulation. Recent experimental evidence has suggested that the CNS-endocrine-immune axis (neuroendocrine-immune axis) proceeds in the direction of the immune response in that the immune system receives signals from the nervous system; in addition, the communication is bidirectional, since the immune system is capable of providing information to the nervous system. This intercellular communication is mediated by products of the immune system including cytokines, growth factors, and neuropeptides made by lymphocytes themselves. Therefore, the distinctions that have been made among lymphokines, growth factors, hormones, and neuropeptides with respect to the organ system in which they function are no longer appropriate.

Both surgical and exercise stress can promote the release of numerous pituitary and adrenal hormones. Thus, it has been suggested that stress can significantly modulate immune function via the endocrine system. Conflicting results have been obtained as to which hormones are secreted in response to stress; however, it is generally agreed upon that these results may be a function of the duration of stress applied along with frequency of the hormone sampling. Studies of the relationship between neuroendocrine peptides and regulation of the immune function have focused on those neuropeptides derived from the polyprotein proopiomelanocortin (POMC), particularly adrenocorticotropic hormone (ACTH) and β-endorphin. Other hormones such as cortisol, growth hormone, prolactin, and the catecholam-

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ines, epinephrine and norepinephrine, have also been central to understanding the relationship between endocrine and immune function. Overall, the evidence for neuronal, endocrine, and immune intersystem communications has formed the basis of numerous studies that have investigated the relationship among psychological stressors, immune function, and the health status of an individual.

**MAJOR LIFE CHANGES AND THEIR EFFECT ON HEALTH AND THE IMMUNE SYSTEM**

Evidence suggests a role for the immune system in the relationship between aversive events and infectious disease. The influence of psychological stressors on general health has been explored in a number of groups of individuals. Overall, individuals who experience stressful life events appear more susceptible to a variety of illnesses. For example, the immunological and pathological consequences of one of the most stressful life events, the death of a spouse, have been the subject for a number of studies, and there is evidence that the widowed survivor experiences an increased risk of morbidity and mortality following the death of their spouse. In addition, other studies have shown that impairments in immune function are associated with bereavement following the death of a spouse, suggesting that immune competence may play a role in their health. Divorce may also result in immunological alterations, particularly in individuals who had been separated for only a short period of time and who had stronger feelings of attachment to their ex-spouse. Similarly, men who had initiated the divorce and who had demonstrated a lower degree of distress and loneliness reported better health than those men who had not initiated the divorce.

There have also been studies of immune function in individuals with major depression. The studies have shown their immune function is lower than non-depressed controls in regard to lymphocyte number and T cell response to mitogen stimulation. One prospective study showed that depressed individuals had a higher incidence of cancer, a finding that may be related to evidence that lymphocytes from high distress individuals have impaired DNA repair mechanisms. Such faulty DNA repair mechanisms have been associated with an increased incidence of cancer. Animal studies have shown that splenic lymphocytes from rats subjected to rotational stress have a depressed ability to synthesize O-methylguanine DNA methyltransferase, an enzyme that is central to the DNA repair metabolic pathway. Since most carcinogens exert their tumorigenic effects via cellular DNA damage, these DNA repair mechanisms may play an important role in the overall integrity of the control of cellular division.

Studies of the effect of chronic stress have also utilized the caregivers of individuals with Alzheimer's disease. The caregivers, because of their provision of long-term care, are typically experiencing long-term or chronic stress. The health impairments that have been reported by these caregivers may be a consequence of diminished immune function. The role of chronic stressors in altering immune status in a negative direction has also been studied in individuals living near a nuclear reactor at which a nuclear disaster had nearly occurred six years earlier.
ACADEMIC STRESS AS A COMMONPLACE STRESSOR

In order to study the relationships among stress, immune function, and infectious disease we have conducted studies on first-year and second-year medical students at The Ohio State University College of Medicine. This group provides an excellent sample to study the effects of an acute cyclical stressor since the medical school curriculum is designed so that the students have either seven or eight two- or three-day major examination blocks throughout the entire academic year. Therefore, the class together as a whole cycles through examination periods, a time generally associated with higher levels of acute stress.

During the last seven years, our laboratory has been involved in characterizing various alterations in immune function, primarily those of the cellular branch of the immune response. The experimental design has consisted of the collection of peripheral blood leukocytes (PBLs) during examination blocks that have been shown to be high distress periods as well as one month before each examination, a time designated as baseline. These PBLs have been analyzed quantitatively in terms of actual cell numbers and for immunological functional capacity.

In general, blood samples obtained from students during the examination periods have shown decreases in the number of natural killer (NK) cells as well as the function of NK cells as measured by cell lysis. Other changes in the cellular immune response that have been associated with stress have included changes in total T lymphocyte number and changes in the percentages of helper and suppressor T lymphocytes. The ability of PBLs obtained from students during the examination periods to proliferate in response to the T cell mitogens concanavalin A (Con A) and phytohemagglutinin (PHA) was compromised as compared to PBLs that were obtained from the same students during the baseline (non-examination) period. The PBLs from students during the examination periods also showed a lowered ability to produce gamma-interferon (\(\gamma\)-IFN) in response to in vitro stimulation with Con A. As an in vivo functional measure of the ability of the cellular immune response to control the latent status of a herpesvirus infection, that is, to control the virus from reactivating and causing recrudescent disease, the antibody levels to the latent herpesviruses cytomegalovirus (CMV), herpes simplex virus (HSV), and Epstein-Barr Virus (EBV) was measured. These studies suggest that during stressful situations, the immune system is less able to control the expression of these virus genomes in latently infected cells, thus allowing the virus to reactivate and stimulate an antibody response. In vitro functional analysis also showed that lymphocytes from those individuals who were EBV-seropositive showed a diminished ability to inhibit the growth of EBV-infected autologous B-lymphocytes when they were obtained during examination periods. The diminished production of PBL-synthesized leukocyte migration inhibition factor (LIF), a lymphokine that is suppressed during HSV-2 recrudescence, was also observed during the examination periods. Lastly, alterations in both plasma and intracellular levels of cyclic AMP (cAMP) were associated with examination stress. This latter observation may provide some insight into the mechanism(s) by which changes in cellular immunity are manifested. Each of these observations will be discussed below in further detail in context of their significance and the possible influence of neuropeptides and other hormones in modulating immune activity.
Natural Killer Cell Activity

Natural killer cells, representing 2 to 5% of PBLs, are leukocytes that do not possess markers of either B cells or T cells and that typically have the morphology of large granular lymphocytes (LGL). Functionally, these cells are able to recognize cell-surface changes on virus-infected cells to which they bind. The NK cells recognize and bind to these target cells in a non-MHC-restricted manner and subsequently lyse them. In addition, NK cells are able to non-specifically kill tumor cells and have been suggested to play a role in the first line of defense against a tumor by immunosurveillance mechanisms. Overall, NK cells are thought to be an important defense mechanism in viral infections and cancer. Activation of NK cells depends on the presence of interferons that are able to transform non-cytolytic NK precursor cells into a lytic state and also enhance the cytolytic capacity of already active cells, thus increasing the number of target cells that can be killed by an NK (effector) cell.

Both the level of NK cell activity and the total number of NK cells have been shown to be diminished in medical students taking examinations as compared to a baseline period in which no examinations were given. The cell numbers were determined by using both a measure of the number of LGL as determined by differential cell count and by the expression of an NK-specific antigen (Leu-7) as measured by the ability to bind to the anti-Leu-7 (HNK-1) monoclonal antibody. It has also been shown that NK cell lysis, using either K562 cells or Molt-4 cells as target cells, was impaired in students during examination periods. This impairment may, in part, be a reflection of the observed decrease in NK cell numbers.

A neuropeptide that may be important for the inhibition of NK cell activity is vasoactive intestinal peptide (VIP). This neuropeptide suppresses human NK cell function as measured by its ability to inhibit NK cell tumoricidal activity in vitro. Although the concentration of VIP used in this study (10^{-7} to 10^{-10} M) was higher than the concentration normally found in peripheral blood (10^{-11} M), it has been surmised that the concentration in the vicinity of VIP-secreting neurons is 10^{-9} to 10^{-10} M. In addition to lymphoid cell acquisition of VIP from the peptidergic innervation of immunologic tissue, it has also been shown that human PBLs and rat mast cells contain immunoreactive VIP. It is not known, however, if these cells actually synthesize VIP or whether they store VIP that is obtained from peptidergic neurons.

In contrast to the immunoinhibitory effects of VIP, the opioid β-endorphin has been shown to enhance NK cell activity over a wide range of concentrations including those concentrations that are believed to be physiologic. To determine the role of β-endorphin in the modulation of immune function in the medical student population, our laboratory plans to measure serum concentrations of β-endorphin at both the baseline and the examination periods.
Interferons, of which there are three (α, β, and γ), are host-coded proteins produced by cells of the body in response to either virus infection (α and β) or contact with specific antigens (γ). γ-Interferon, also known as “immune” or type 2 IFN, is produced by non-sensitized lymphocytes in response to mitogens and by sensitized lymphocytes when stimulated with specific antigens. All three IFNs are known for their antiviral activity by interfering with virus replication. In addition, IFNs possess the ability to inhibit cell growth, with tumor cells being more sensitive than normal cells. Interferons also have potent effects on the immune system in both an inhibitory and enhancement fashion. For example, as stated above, IFN is a major regulator of NK cell activity. Early murine studies suggest that there might be a nervous system component involved in the synthesis of IFN. Stress-related changes in IFN production were shown in virus-infected mice that had been subjected to physical stressors such as shock. Stress may not only modulate IFN synthesis but also other immune functions, such as macrophage tumoricidal activity, which is dependent on IFN stimulation.

We have examined the ability of PBLs to synthesize IFN after stimulation with Con A. In two separate studies with two different classes of medical students, we found a significant decrease in the ability of PBLs obtained during examination periods to synthesize γ-IFN. The downregulation of γ-IFN production has broad implications for T cell and NK cell function and could in part be associated with the decrements observed in the cellular immune response as demonstrated by work from our laboratory and others.

The role of neuropeptides in the production of γ-IFN in vivo is not well characterized. However, in vitro studies have shown that arginine vasopressin (AVP), a posterior pituitary nonapeptide, can regulate immune function by providing a helper signal for induction of γ-IFN. AVP also plays a role in the stress response by direct stimulation of release of ACTH and the enhancement of ACTH release by corticotropin-releasing factor (CRF). The ability of AVP to induce γ-IFN production and to also stimulate release of ACTH is somewhat paradoxical considering that ACTH can inhibit γ-IFN production by interfering with helper cell function. Another feature of AVP is its ability to enhance learning and memory by its functioning as a neurotransmitter. Therefore, it is interesting to speculate what the relationship is, if any, between examination stress, the secretion and utilization of AVP, and the immune response.

Total T Lymphocytes and T Lymphocyte Subpopulations

Lymphocytes are produced in the primary lymphoid organs (thymus and adult bone marrow) at a rate of 10^9/day. Some of these cells migrate via the circulation into the secondary lymphoid tissues, namely, the spleen, lymph nodes, and unencapsulated lymphoid tissue. These circulating lymphocytes include two distinct populations of cells, one of which is large and contains azurophilic granules. The other population is characterized by cells that are small and that possess a high nuclear-to-
cytoplasmic ratio. This latter population contains both the B cells and the T cells that comprise the humoral and the cellular branches of the immune response, respectively. The T cell population contains three basic subsets: helper cells, suppressor cells, and cytotoxic cells. The helper cells play critical roles in mediating the B lymphocyte proliferation and differentiation sequence that is critical for the synthesis of immunoglobulins. They also play an important role in the overall development and function of the cytotoxic/suppressor cell population along with the interactions between other T lymphocytes and macrophages. Therefore, in the absence of helper cells, severe immunodeficiency may result as occurs in AIDS.

In the medical student study described above, we were interested in determining if academic stress could alter the percentages of total T lymphocytes, helper T lymphocytes (Th), suppressor T lymphocytes (Ts), and cytotoxic T lymphocytes (Tc) that are normally observed in the peripheral blood. Quantitation was possible by the use of monoclonal antibodies (Ortho Diagnostics) OKT-3 (total T), OKT-4 (Th), and OKT-8 (Tc/Ts) using fluorescent flow cytometry. In one study we found that medical students experiencing examination stress had significant decreases in the percentages of OKT-3+ T lymphocytes, OKT-4+ lymphocytes, and OKT-8+ lymphocytes. It is interesting that the mean percentage of OKT-3+ lymphocytes even at the baseline period were below the normal range that is obtained with the OKT-3 antibody. This may be due to the fact that the students were already exhibiting some immunologic changes, since they had already gone through most of the academic year and had experienced a number of high- and low-level stressful periods.

Mitogenic Responsiveness

Mitogenic lectins such as Con A and PHA are derived from the jack bean and kidney bean Phaseolus vulgaris, respectively, and are used to polyclonally (non-specifically) stimulate T cells. These mitogens are proteins that bind and crosslink specific cell surface carbohydrate determinants. It is these mitogens that cause a resting T cell to synthesize RNA, protein, and DNA, leading to the development of large lymphoblasts that may then divide. The ability to quantitate such a mitogenic stimulation in terms of lymphocyte proliferation as measured by radioactive thymidine (tritiated thymidine) uptake and incorporation into DNA is a semiquantitative functional test of T cell activity that is believed to mimic the series of events that occur in vivo following stimulation of lymphoid cells with a specific antigen.

In the medical student studies, the PBLs from students taking examinations showed a diminished responsiveness to Con A and PHA as compared to the baseline. These results suggest that, in vivo, the ability to manifest an antigen-specific cellular immune response may be compromised. Our laboratory is currently investigating the proliferative capacity of these PBLs in response to their binding to an anti-T3 antibody. The T3 surface glycoprotein (molecular weight = 20K) is associated with the T cell receptor and is involved in T cell activation. Binding of this anti-T3 antibody results in mitogenicity for resting T cells and, although the stimulation is not antigen-specific, it is a measure of the capacity of resting T cells to proliferate in response to stimulation of the T cell receptor by antigen binding.

Speculation on the role of neuropeptides in mediating these mitogenic processes of lymphocytes leads to some interesting hypotheses. For example, substance P, an
11 amino acid peptide that is widely distributed in peripheral sensory nerves that innervate organs and tissues, stimulates T cells to proliferate and enhances the activity of T cell mitogens.\textsuperscript{55,56} In addition, direct binding studies have demonstrated the presence of vasoactive intestinal peptide (VIP) receptors on cells of lymphoid origin.\textsuperscript{57,58} Like substance P, VIP also affects the proliferative responses to T lymphocytes. However, whereas substance P is stimulatory, VIP has been shown to be inhibitory. Murine studies have shown that VIP is able to inhibit the mitogen-stimulated proliferation of cells from the spleen, Peyer's patches, and mesenteric lymph nodes.\textsuperscript{59} \(\beta\)-endorphin also has been shown to be a potent inhibitor of human lymphocyte proliferation, specifically, in response to treatment with PHA.\textsuperscript{60,61} However, some studies have also shown that \(\beta\)-endorphin can enhance lymphocyte proliferation.\textsuperscript{62} The presence of both classical and non-classical opiate receptors on lymphocytes that bind \(\beta\)-endorphin\textsuperscript{63} indicates that \(\beta\)-endorphin may play an important role in immune function.

Pituitary hormones such as growth hormone, also known as somatotropin, and prolactin have the potential to regulate important immune functions. For example, experiments in which spleen cell proliferation (as a result of mitogenic stimulation) can be inhibited by anti-rat prolactin antiserum indirectly demonstrate the potential role of prolactin as an immunoenhancing agent. This is even more important in light of the finding that spleen cells that are stimulated with Con A can produce immunoreactive prolactin.\textsuperscript{64} In contrast, the binding of somatostatin to lymphocyte receptors activates an inhibitory guanine nucleotide binding protein that, in effect, can inhibit the signal mediated by the VIP receptors. As a consequence, somatostatin has been shown to significantly inhibit PHA stimulation of human T lymphocytes\textsuperscript{65} and proliferation of both spleen cell--derived and Peyer's patches--derived lymphocytes.\textsuperscript{56} Other \textit{in vitro} studies have demonstrated the presence of somatostatin receptors as evident by the ability of somatostatin to inhibit T cell responses.\textsuperscript{66-69} There is also evidence that thymosin peptides, which are produced by cells within the thymus and perhaps by neuronal cells,\textsuperscript{70} may also play a role in altering the mitogenic capacity of lymphocytes by augmenting their proliferative responsiveness. Support for the hypothesis that thymosin peptides have a direct role in immunomodulation is the finding that T lymphocytes have receptors for these peptides.\textsuperscript{71-73}

\textit{Control over Latent Herpesviruses}

Stress-induced changes in the competence of the cellular branch of the immune system are thought to be responsible for enhanced reactivation and expression of latent herpesviruses as measured by elevated antibody titers to such viruses. In individuals with immunosuppressive diseases or who are receiving immunosuppressive therapy, there are often elevated antibody levels to herpesviruses, and in some instances, shedding of infectious virus. These elevated antibody levels are thought to occur in response to the increased production of viral antigens. Once the cellular immune response regains competency and is able to maintain control over the induction of the latent virus genome and subsequent virus protein synthesis, the antibody titers to the respective antigens decrease. Overall, it is the suppression of the cellular immune response that plays an important role associated with the observed higher antibody titers.
We have shown that there are increased serum antibody levels to three herpesviruses, EBV, HSV-1, and CMV, associated with academic stress. These viruses all establish a latent infection following the initial primary infection. In one study, these increases were based on comparisons with antibody titers that were observed upon the students' return following summer vacation. For example, the geometric mean titers (GMT) to EBV virus capsid antigen (VCA) IgG following summer vacation were consistent with what has been observed in seroepidemiological studies of the adult North American population in which the EBV VCA GMT was found to be 1:80. During examination stress, these titers were greater than 1:640. The students' self-reported distress data indicated that their highest levels of distress occurred during final examinations, while their lowest levels were immediately upon their return to school following summer vacation. In addition, students who had reported greater loneliness had higher titers to the EBV VCA. A subsequent study that was conducted during the academic year showed similar examination stress–related increases in EBV VCA antibody titers as compared to titers observed during the baseline period one month before examinations. Overall, these results suggest that under conditions of high stress, the cellular branch of the immune response is compromised to a level whereby reactivation of latent herpesvirus may occur with a concomitant increase in antibody titers. These studies, which also show that psychosocial risk factors can result in an increase in EBV reactivation, support similar findings by Kasl et al. in which West Point cadets who experienced psychosocial stress showed an increased risk for EBV seroconversion and higher antibody titers to EBV in those individuals who seroconverted in the absence of clinical symptoms.

**EBV-specific T Cell-Mediated Killing**

The ability to exert control in preventing recrudescence EBV infections and subsequent increases in antibody titers may rely on the efficacy of the T cell-mediated component of the immune response. EBV reactivation may occur despite the presence of a competent immune system; however, the expression of early virus-specific antigens such as the lymphocyte determined membrane antigen (LYDMA) on the surface of the latently infected cells may provide a target for cell-mediated lysis that is carried out by a population of EBV-specific memory T lymphocytes. Therefore, the presence and function of such a lymphocyte population may play an important role in the control of recurrent EBV infection.

As a measure of the T cell component of the EBV-specific immune response in the EBV-seropositive subset of the medical student population described above, an in vitro assay, first described by Moss et al. and Rickinson et al., was conducted. Briefly, PBLs from the students were infected with EBV and cultured in vitro for one month. During this time period, the cultures were examined for the presence of morphologically growth-transformed cells. A decrease in the number of cultures showing such transformed cells indicated that the progenitor EBV-transformed cells (EBV-infected autologous B lymphocytes) that are ultimately responsible for the establishment of growth-transformed lymphoblastoid cells have been destroyed,
presumably by the in vitro-stimulated EBV-specific cytolytic T memory cells that were initially present in the mononuclear cell preparation. The results of these studies demonstrate that the mononuclear cells obtained from students during the examination period exhibited a diminished level of EBV-specific cell killing as compared to those cells obtained from students during the baseline period. As expected, mononuclear cells from EBV-seronegative individuals were unable to inhibit the outgrowth of EBV-transformed autologous B lymphocytes. Overall, these results suggest that academic stress can downregulate specific T cell killing to EBV-infected cells and perhaps to other virally infected cells. However, it is not known whether these observations are a function of the ability of the EBV-specific memory cells to be restimulated in vitro, and presumably in vivo, or whether they represent a decrease in the lytic activity of those cells that have been restimulated.

Leukocyte Migration Inhibition Factor

Leukocyte migration-inhibition factor (LIF), is a protein with a molecular weight of 68,000 that inhibits the migration of polymorphonuclear leukocytes but not monocytes. The ability of PBLs from HSV-2 seropositive individuals to produce this lymphokine in vitro in response to HSV-specific stimulation is suppressed during the recrudescence of HSV-2 infections as compared to the levels produced by PBLs from individuals during the convalescent phase of HSV infection. Moreover, Sheridan et al. have shown that these depressed levels of virus-specific cell-mediated immunity as measured by in vitro HSV-specific LIF production correlate with a significant increase in T8+Ia+ cells that appear to possess suppressor activity.

As discussed above, increased antibody titers to HSV have been associated with academic examination stress, presumably due to virus reactivation. As one measure of the cellular component of the immune response to HSV infection, we have examined the ability of the PBLs from HSV-seropositive medical students to produce LIF in response to HSV-specific stimulation. It was shown that PBLs from medical students who were undergoing examinations produced lower levels of MIF as compared to the PBLs from the same students during the baseline, non-examination, period. Thus, the apparent relationship between HSV reactivation and deceased LIF production in the medical student population is consistent with the earlier findings that linked recrudescent infection with LIF activity and the data on reactivation of latent EBV.

As another measure of the modulation of the HSV-specific cell-mediated immunity in the HSV-seropositive medical student population during examinations, our laboratory is currently investigating the ability of HSV-specific memory lymphocytes from HSV-seropositive students to proliferate in vitro in response to the presence of ultraviolet (UV) light-inactivated HSV virions. It has been previously demonstrated that PBLs obtained from individuals during recrudescence, convalescence, and quiescence all exhibit similar HSV-specific lymphoproliferative responses. In lieu of these observations, it may be possible to explain any observed differences in lymphoproliferative capacity between examination period and the baseline period on the basis of a deficiency in some aspect of the memory immune
response. Because the HSV antigens must be processed by macrophages to stimulate the HSV-specific lymphocytes, these differences could also be a function of defects in either macrophage processing or presentation of the antigen as well as the ability to stimulate other cell types via the secretion of IL-1. Our laboratory is currently examining the ability of macrophages obtained from students during the baseline and examination periods to produce IL-1. In the event that such a deficiency in HSV-specific proliferation is found to be associated with examination stress, further studies will be necessary to delineate the mechanism(s) by which this deficiency is manifested.

**Plasma and Intracellular Levels of Cyclic AMP**

Cyclic adenosine monophosphate (cAMP) is a low molecular weight substance that is a key element in many control systems, including its action as a second messenger after the binding of many hormones to their respective receptors. The importance of cAMP in immune function was demonstrated by Coffey and Hayden, who showed that cAMP is able to inhibit lymphocyte proliferation and cytotoxicity, lymphokine and antibody production, and cell motility. Although there have not been studies linking long-term psychological stress with increases in cyclic nucleotides, it is possible that β-adrenergic stimulation, which has already been shown to be associated with increased levels of cAMP during acute stress, may induce an increased synthesis of cAMP during such long-term stress. Because cAMP may indeed be involved in suppression of immune function, this cyclic nucleotide was examined in the medical student examination studies as a possible mediator in the suppression of the immune function.

The levels of cAMP were determined by radioimmunoassay in both the plasma and in the PBLs from medical students at both the baseline (non-examination) and at the examination periods as described by Glaser et al. Although there were no differences in the levels of plasma cAMP between the first baseline (September) and examination (October) periods in the academic year, there was an increase in cAMP between the second baseline period (January) and the second examination period (February). In fact, the levels of plasma cAMP during the January baseline period were greater than during the September baseline period. Examination of PBLs for intracellular levels of cAMP revealed that the levels of cAMP in cells obtained from students under examination stress were significantly greater than those cells obtained during the baseline period.

Although the exact role(s) of cyclic nucleotides such as cAMP in modulating immune function is (are) not known, it is likely that their function is through their ability to activate a protein kinase resulting in the phosphorylation of some cellular protein or proteins. There has been speculation as to the substances that mediate such a rise in intracellular cAMP. As discussed above, VIP can act on VIP receptor-bearing T lymphocytes resulting in the activation of adenyl cyclase and the subsequent accumulation of cAMP. Thus, the increased levels of cAMP observed in both the plasma and in the PBLs of medical students during examination may, in part, be due to VIP.
Apoptosis (Programmed Cell Death)

The programmed deletion of cells is a widespread phenomenon in biology and is particularly an important component in embryonic development, tissue modeling, normal cell turnover, and endocrine-induced atrophy. As a subset of this process of normal cell turnover is the death and removal of those cells that have undergone some exposure to toxic stress or trauma. One mechanism by which such damaged cells die is simply via necrotic death that is associated with the structural and biochemical loss of cell integrity. Another mechanism involves programmed cell suicide known as apoptosis in which an early endonucleolytic fragmentation of duplex DNA occurs presumably as a consequence of specific gene expression.

Apoptosis is believed to function in the protection of organisms from the accumulation of cells with induced heritable changes in cellular DNA, including those induced by cytotoxic insults such as exposure to ionizing radiation, chemical toxicity, and virus infection. However, exposure of these cells to phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) has been shown to "protect" the cells that show abnormal DNA fragmentation and to block cell death. The mechanism by which this process is mediated remains unknown. The ability of macrophages to bind apoptotic thymocytes in preference to non-apoptotic cells suggests that macrophages may play a key role in the removal of other cells undergoing apoptosis in vivo.

Apoptosis was studied in the PBLs of medical students experiencing examination stress. The PBLs were exposed to a lethal dose of gamma radiation (10 Gy) either in the presence or absence of TPA and then cultured for 96 h. At the end of this time period, cell survival was measured by the level of total DNA, and mitogenic stimulation was determined by the incorporation of [3H]dThd (2-deoxyriboside). TPA treatment of both irradiated and non-irradiated PBLs from students undergoing examination stress resulted in significantly greater levels of total cellular DNA and increased levels of DNA synthesis as compared to those cells obtained from students during the baseline period. These studies demonstrated that physiological consequences of psychological stress have a profound effect on the ability of a phorbol ester to inhibit radiation-induced cell death. These alterations in the ability of immune cells to express apoptosis support the concept that psychological stress may impair the function of the immune system. Most likely, the stress-related physiological changes are manifested via the endocrine system though this remains to be proven. In fact, if this relationship between stress, endocrine function, and apoptosis is correct, then the physiological changes that result from psychological stress could lead to an enhanced responsiveness to apoptosis inhibitors such as endogenous growth factors. The inhibition of apoptosis in PBLs could also lead to reduced immunocompetence and, consequently, a greater risk of infectious disease. If such a psychologically induced inhibition of apoptosis also occurs in other cells of the body, then one would expect a progression of errors to occur within cell genomes leading to an increased probability of cell survival, and a subsequent increase in the risk of developing an environmentally associated malignant disease. It is for this and other reasons that it has been suggested that the consequences of the inhibition of apoptosis may be indistinguishable from those of direct mutagenesis. The observation that individuals subjected to stress exhibit lower levels of NK cell activity could be interpreted as enhancing the possibility of the initiation of cell transformation and
the outgrowth of tumor cells. Because macrophages play an important role in recognition and binding to apoptotic cells, any stress-related impairment in macrophage function could also lead to a decreased clearance of these damaged cells.

HEALTH-RELATED CONSEQUENCES OF THE CNS-ENDOCRINE MODULATION OF THE IMMUNE SYSTEM

Although interactions have been demonstrated among the CNS, endocrine system, and the immune system, it is the functional capacity of the immune system and its ultimate effect on the health of an individual that is perhaps the most important facet in the study of psychoneuroimmunology. There is a particular interest in examining this interrelationship from the perspective of behavioral immunology by exploring the hypothesis that various psychological stressors have an impact on the CNS with subsequent effects on the endocrine system and modulation of the immune response. It is through these relationships that psychological stressors are thought to influence the health of individuals.

It is quite clear that large deficits in immune function such as those associated with congenital and acquired antibody deficiency disorders (X-linked agammaglobulinemia) and cellular immunodeficiency disorders (DiGeorge's syndrome, AIDS) are associated with a significant increase in morbidity and mortality. In general, individuals with antibody deficits are especially susceptible to pyrogenic infections (pneumococci, streptococci) despite their ability to cope normally with fungal and viral (except enterovirus) infections. In contrast, those individuals with cellular immune deficits are particularly prone to certain bacterial and protozoal infections caused by organisms such as Mycobacterium tuberculosis, Pneumocystis carinii, and Pseudomonas aeruginosa. Despite these known relationships between severe deficits in immune function and health risk, it is not known what deviations from the mean level of immune competence are necessary to result in an increased risk of pathology. Likewise, the kinetics of the immune response in relationship to disease risk is unknown. It should also be noted that behavioral differences between normal and distressed populations may also contribute to an increased incidence in health problems by affecting the immune system by a mechanism that is independent of the neuroendocrine-immune axis. For example, differing lifestyles of individuals in the distressed population as compared to non-distressed individuals may have immunological consequences. In a study of marital disruption, it was suggested that non-married individuals have a greater tendency to experience riskier lifestyles (e.g., alcohol consumption, tobacco and drug use, poorer nutrition, and sleep deficiency) than married persons. Such lifestyles may be responsible for some further impairment of the immune system. In several laboratories, long-term, longitudinal studies that are examining the relationship between psychological stress, the immune system, and health status should provide further insight into this area.

There have been a number of studies that have shown that individuals who have experienced major negative life events are at a greater risks for a variety of illnesses. For example, in studies of separated, divorced, and widowed individuals, it has been shown that although all these groups of individuals experience a lower health status, a greater risk for illness is associated more with separation and divorce than with
bereavement. In addition, in one study separated and divorce men were not only more distressed and lonelier than married men but that they also experienced a significantly greater number of illnesses. Furthermore, those men who had separated or divorced within the last year and who had initiated the separation were less distressed and reported better health status than the noninitiators.

As discussed above, there is evidence that medical students who are experiencing academic (examination) stress experience some deficits in their immune function. The fact that these students also self-reported a higher incidence of infectious disease symptoms (particularly symptoms associated with upper respiratory tract infections) during the examination periods suggests that there is possibly a link between stress-related immunosuppression and health in this population. Future long-term longitudinal studies must be performed to answer this important question.

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ANNALS NEW YORK ACADEMY OF SCIENCES


