Stress-Associated Immune Suppression and Acquired Immune Deficiency Syndrome (AIDS)

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Studies from several laboratories have shown that a variety of stressors downregulate the immune response in animals as well as humans. Several components of the cellular immune response have been shown to be involved in this association, including the ability to control and maintain latent herpesviruses. It has now been shown that the oncogenic retrovirus (HTLV-III/LAV) associated with acquired immune deficiency syndrome (AIDS) can latently infect human T lymphocytes. The data obtained from studies on neuroimmunodulation may have implications for the clinical course of immune suppression associated with AIDS.

It has now been established that there are multiple mechanisms whereby the central nervous system (CNS) and the immune system interact with each other. For example, it has been shown that there are nerve endings in different lymphoid organs, such as the spleen and lymph nodes. The endocrine system has also been implicated as a mediator between the CNS and the immune systems; certain hormones such as cortisol can regulate certain aspects of cellular immunity (9, 25). In addition, it has been possible to demonstrate receptors on the surface of lymphocytes to hormones such as ACTH, and lymphocytes may be able to produce certain hormones as well (32). Thus, the demonstration of neuroimmunomodulation in animals and humans may partially explain anecdotal data suggesting that certain kinds of stressors may increase risk for a variety of infectious diseases, and perhaps even cancer, providing some legitimate scientific evidence. The study of “psychoneuroimmunology” has included the examination of the immune response in association with severe stressors such as depression and bereavement, and decrements in cellular immunity have been demonstrated (1, 35, 54).

It has now been shown by several laboratories that a variety of stressors can modulate cellular immunity. It has been hypothesized that stress-related alterations in immune function may be associated with increased susceptibility to infectious
disease and perhaps to cancer. Previous work from our laboratory has been concerned with studying the effect of commonplace stressors on cellular immune function. For example, we have found reproducible significant decrements in natural killer (NK) cell activity using two different target cells in three different medical student studies in which we examined the impact of academic stress on immunity. We have also found a decrease in the percentage of NK cells using two independent measures. Similar reliable immunological changes found associated with examinations were also found in the total number of T lymphocytes as well as in changes in the percentages of subpopulations of T lymphocytes, mitogen responsiveness, interferon (IFN) production by peripheral blood lymphocytes (PBLs) stimulated with concanavalin A (Con A), and the ability of the cellular immune response to be modulated in connection with the expression of three latent herpesviruses (17, 23, 36, 37).

CHANGES IN CELLULAR IMMUNITY ASSOCIATED WITH PSYCHOLOGICAL STRESS

Over the past several years, our laboratory has been involved in studying different aspects of cellular immunity and the impact of a variety of psychological stressors on the maintenance of the cellular immune response, including percentages of subpopulations of lymphocytes. Blood samples were obtained at a base-line period approximately 1 month prior to a block of examinations at The Ohio State University College of Medicine in the first 2 years of the curriculum. The examinations in different specialties are given all together over a 1- to 3-day period throughout the academic year; thus, the medical class in this program cycles together as a group through examination periods. We have taken advantage of this arrangement to study the effects of academic stress on immunity and health. The cellular immune response was studied by testing for quantitative and qualitative changes in lymphocytes obtained from base-line blood samples as well as from blood samples obtained on the day of examinations. In addition, self-report data were also obtained in order to determine if there were changes in psychological stress associated with taking examinations. The results obtained from multiple studies with first-year and second-year medical students are reproducible and have demonstrated that there is a decrease in the mitogenic response to both Con A and phytohemagglutinin (PHA) by lymphocytes obtained on the day of examinations. We have also found that there was a decrease in NK lysis and in the percentages of OKT-4⁺ (helper) T lymphocytes and OKT-8⁺ (suppressor) lymphocytes under these circumstances as well as changes in total T lymphocytes (OKT-3⁺). The reduction in NK cell lysis was not dependent on the target cell used in the assay (17, 36, 37).

In a separate study, we examined the mitogenic response of lymphocytes obtained from inpatient psychiatric patients (nonmedicated) as well as NK activity. The patient population was divided into high- and low-loneliness groups using the UCLA Loneliness Scale. We found that the patients in the high-loneliness group had a poorer mitogenic response to PHA as well as poorer NK lysis when compared to
the patients in the lower-loneliness group (38). These data are consistent with data from our studies on academic stress.

EFFECT OF STRESS ON THE MAINTENANCE OF LATENT HERPESVIRUS

There has been speculation that links stress and the appearance, duration, and intensity of herpesvirus infections with the presumption that these changes reflect alterations in cellular immunity. For example, it was found that there was an increased risk for infection by Epstein–Barr virus (EBV) in West Point cadets associated with certain psychological risk factors: high levels of motivation, poorer academic performance, and having a father who was an overachiever (33). In another study, Luborsky et al. (41) found that unhappiness among nursing students was a predictor for herpes labialis lesions.

The role of immunity in controlling the recurrence of latent herpesvirus infections is not well understood. Normally, after an active herpesvirus infection, the virus is repressed in a latent state in certain host cells. It is common that in individuals who become immune suppressed, either naturally by infection with other viruses or, for example, after radiation therapy in cancer patients, there is often an increase in antibody titers to one or more of the herpesviruses, including herpes simplex virus type 1 (HSV-1), EBV, and cytomegalovirus (CMV). These antibody increases are presumably caused by the humoral immune response to the increase in virus-specific antigens after reactivation. It is thought that the cellular immune response is very important for the limitation of the primary infection and controlling the expression of latent EBV (26,39,51). Animal studies have shown that induced immune suppression can reactivate latent HSV (45,46).

We have attempted to assess possible stress-related changes in herpesvirus antibody titers using a prospective design. We have studied the effect of academic stress on the maintenance of EBV, HSV, and CMV. Any changes observed in antibody titers to any one or all of these herpesviruses would presumably reflect modulation of latent virus genomes by the cellular immune response. In one study, we examined a total of 49 EBV-seropositive medical students for changes in EBV antibody titers that could be related to academic stress. The average age of the 16 women and 33 men was 23 years. The first blood sample was obtained 1 month before final examinations, at the end of April. A second sample was obtained the first day of final examinations, at the end of May, and a third blood sample was collected during the first week of September, after the students had returned from vacation. As in all our studies, blood was obtained at the same time during the day (in the middle of the day) to avoid diurnal variations. Self-report data were also obtained at the time the blood was drawn.

The self-report measures used in this study included the Brief Symptom Inventory (BSI), a short form of the Symptom Checklist-90 (10). We also used the UCLA Loneliness Scale (52) to provide a brief subjective measure of the overall adequacy of interpersonal contacts. The EBV virus capsid antigen (VCA) mean antibody titers
(GMT) were obtained using the indirect immunofluorescence test. Antibody titers to HSV and CMV were obtained by ELISA. The results of the BSI for the three sample points confirmed that the examination sample was associated with the most distress, followed by the base-line sample, with the lowest self-ratings of distress occurring after the students’ return from summer vacation. We found a significant change in EBV VCA antibody titers across the three sample points. We also found that high-loneliness subjects had significantly higher VCA titers than low-loneliness subjects. The interaction between loneliness and change across sample points did not reach significance. Similar changes were also observed for antibody titers to the EBV early antigen (EA). When antibody titers were assayed for HSV and CMV from the same blood samples, similar fluctuations were observed (16).

In order to evaluate the possibility that there were fluctuations in IgG antibody titers to other antigens as well, rather than specifically to the three latent herpesviruses, we measured antibody titers to poliovirus type 2 as a recall antigen. The widespread use in school vaccination programs provided some assurance that most students would have antibody to this virus. We did not find any significant change in antibody titers to poliovirus type 2 in the same samples in which we found changes to the three herpesviruses, supporting the hypothesis that the herpesvirus data reflect stress-related changes in virus latency. In addition, in order to evaluate the possibility that there was a subclinical epidemic of EBV infections in our medical students at the time the study was taking place, we measured antibody titers to EBV VCA IgM. All plasma specimens were found to be negative for EBV VCA IgM antibody, suggesting that the results that we obtained were not caused by an epidemic of EBV infections in this group (16).

It has been shown, both in humans and in laboratory animals, that the immune function generally declines with age. For example, it is known that T-lymphocyte-mediated immune function for the production of certain autoantibodies, such as antinuclear antibodies, tends to increase with aging (2, 49, 50, 58). There are also data that suggest that although there are no changes in levels of IgM in plasma, there is an increase in IgG and IgA in older individuals (49). In order to examine another aspect of cellular immunity and its control of the latent EBV genome under natural conditions in otherwise normally healthy individuals, we examined EBV antibody titers in a geriatric population (24). We assayed levels of VCA IgG and IgA as well as EA IgG in a geriatric population in order to determine if there were any differences in the antibody patterns to these antigens when compared to a younger population, i.e., our medical students. We found that 89% of the geriatric blood samples were positive for EA IgG, and 83% of the medical students were positive for EA IgG. One hundred percent of the geriatric blood samples were positive for VCA IgG, and 87% of the medical students had antibodies to VCA IgG. We found that approximately 7% of the plasma samples obtained from the medical students were positive for VCA IgA antibody; however, 36% of the samples obtained from the geriatric population were found to be positive for this antibody. Statistical analyses of these data show that the EA GMT in the medical students is significantly lower than the EA GMT of the geriatric group, as determined using
analysis of variance. A similar statistically significant difference was found between VCA IgG GMTs in the two populations as well. A statistically significant difference was found in VCA IgA levels.

At no time during the course of the study did any one of the geriatric participants complain of symptoms compatible with infectious mononucleosis or present with persistent EBV infection, and the higher levels of antibody in the geriatric group were not associated with clinical disease. The data suggest that there may be some loss of control over the latent EBV genome in geriatric individuals, presumably because of a less efficient cellular immune response. This could be related, for example, to a depression in T-lymphocyte-mediated immune function, already discussed. These data are consistent with the data obtained with the medical students, suggesting that negative modulation of the cellular immune response, either with aging or because of psychological factors such as academic stress, can allow at least some reactivation of latent herpesviruses.

DEPRESSION IN THE SYNTHESIS OF INTERFERON ASSOCIATED WITH ACADEMIC STRESS

There is good evidence that NK cell activity has an important role in the immune response to viral infections and perhaps cancer (27,28). Interferon is a major regulator of NK cell activity. It can activate the lytic activity of target binding cells, enhance cytolysis of target cells, and increase the number of target cells that then can be killed by an effector cell. In addition, there is also evidence that NK cells themselves can produce IFN (27,28).

In studies with rodents, it was suggested that there may be CNS mediation of IFN synthesis. Stress-associated changes in IFN production were found in virus-infected mice after the mice were exposed to physical stress such as shock (7,31). In addition, various stressors might also reduce responsiveness of certain immune functions to IFN stimulation. It was found that from 52 to 75% reduction in macrophage tumoricidal function in interferon-treated mice was observed following restraint (47).

As discussed earlier, in two studies from our laboratory we found a significant decrease in NK cell activity using both K562 cells and MOLT-4 cells (23,36). In the same studies, we measured changes in total IFN production by Con-A-stimulated PBLs taken at base line and at the time of examinations. We also determined the number of NK cells using two different protocols: the Leu-7 monoclonal antibody that recognizes a surface marker on NK cells and the number of large granular lymphocytes (LGLs), the phenotype of NK cells. As in previous studies, self-report data were obtained along with the immunological data. Again, we were able to document significantly increased distress associated with examinations, in comparison to base line. We found that the production of IFNs by Con-A-stimulated lymphocytes declined sharply from the first (base-line) to the second (examination) samples (23). We also measured IFN levels in plasma from both blood samples and
found that no measurable levels could be detected in either sample point, as expected. These data have been confirmed in a second study in which medical students were followed over an entire academic year (three base-line and three examination periods). The ability of Con-A-stimulated PBLs to synthesize γ IFN was markedly inhibited during the examination periods as compared to base-line controls (22). We found that the percentage of anti-Leu-7⁺ NK cells declined significantly in the PBL samples obtained during examinations as compared to the base-line sample. Similar results were obtained in the percentage of LGLs; NK cell lysis also decreased significantly, confirming previous studies.

The data obtained in these studies demonstrate a very large and significant decrease in the amount of γ IFN produced by Con-A-stimulated PBLs obtained from medical students during examinations in contrast to the base-line values obtained 6 weeks earlier (23). There was also a significant decrement in the activity of NK cells as represented by lysis of MOLT-4 target cells, and a decrease in the number of NK cells (percentage Leu-7⁺ cells and percentage LGLs) was also found. Similar results were obtained when the absolute number of Leu-7⁺ NK cells for each person and for each effector-to-target-cell ratio were calculated. These data suggest that the decrease in NK cell lysis reported earlier in animals and in studies from our laboratory may be caused at least in part by a decrease in the total number of NK cells (22). These data may have important health implications, particularly in regard to AIDS, and are discussed below.

CONTROLLING FOR OTHER FACTORS THAT COULD IMPACT ON CELLULAR IMMUNITY

In all of our studies, we have determined if other factors could have been implicated in the decrease in the cellular immune response. For example, we have determined that sex, age, loss of sleep, change in weight, smoking, alcohol, and caffeine intake in the medical student studies were not associated with any of the immunological changes we observed. Since poor nutrition has been shown to impact in a negative way on the immune response, in several of our studies we examined this possibility by using protein assays to obtain general nutritional status, for example, serum albumin and transferrin levels. These two protein markers have half-lives of approximately 20 days and 8 days, respectively, giving us a range of nutritional data. Serum proteins tend to decrease in protein malnutrition, and it has been shown that the serum transferrin levels can be used to assess the effectiveness of total parenteral nutrition (34). In all studies in which we measured these protein markers thus far, all participants were within normal ranges for albumin and transferrin.

LABORATORY STUDIES ON THE REACTIVATION OF LATENT EBV

The EBV is a human oncogenic herpesvirus that infects and becomes latently associated with human and certain nonhuman B lymphocytes and has also been
detected in the epithelial cells of nasopharyngeal carcinoma (NPC) tumors. We have been interested in studying the expression and regulation of the EBV genome in nonlymphoblastoid cell lines, for example, epithelial/lymphoblastoid and NPC/epithelial hybrid cells, as a model for studying NPC, since there are no EBV-genome-positive NPC tumor cell lines. Thus far, it has been possible to obtain several EBV-genome-positive epithelial/lymphoblastoid and epithelial/epithelial hybrid cells (18–21,56,57). The latent EBV genome in these cells is generally in a repressed state; i.e., only the nuclear antigen (EBNA) is expressed, and EA and VCA are not synthesized. However, several years ago we found that by using iododeoxyuridine (I UdR), hybrid cells such as D98/HR-1, which contain a repressed EBV genome, can be induced to synthesize EA, VCA, and virus particles (15,19,21). It was of interest to us to determine whether the EBV genome latently associated with NPC epithelial tumor cells could also be induced to replicate in a similar way. Explant cell cultures were prepared from NPC biopsies and treated with IUdR and examined for the reactivation of the latent virus genome. We were able to demonstrate that, similar to the D98/HR-1 hybrid cells, the EBV genome in NPC tumor cells could be induced to synthesize at least EA after treatment with IUdR, suggesting that the association between the EBV genome and cell genomes in both the hybrid cells and NPC tumor epithelial cells may be the same and that the mechanisms whereby IUdR induces the endogenous genome may also be similar (14).

THE NEUROENDOCRINE AXIS AS A LINK BETWEEN THE CNS AND THE IMMUNE SYSTEM

It is not within the purview of this report to define stress. Suffice it to say that if the distress associated with taking examinations (academic stress) is sufficient to affect cell-mediated immunity, then the stress associated with the social interactions of the gay community could have immunological consequences. It has been shown in many studies that physiological stress results in changes in blood levels of a variety of hormones. It is thought that response to emotional stress is initiated in the hypothalamus, which ultimately modulates the release of pituitary hormones, as reviewed by Borysenko and Borysenko (5). Catecholamines are released and initiate a secondary cascade of hormonal effects. As demonstrated, corticosteroids and catecholamines are associated with stress; however, additional hormones such as growth hormone, somatotropin (6), adrenocorticotropic (43), melanocyte-stimulating hormone (29), prolactin (44), thyrotropin (11), vasopressin, aldosterone, calcitonin, parathyroid hormone, thyroxin, glucagon, renin, erythropoietin, and gastrin also have been found to be associated with stress (8). The impact of changes in blood levels of these hormones on the immune response in animals is reviewed by Landsberg (40). These changes include progressive atrophy of lymphoid organs, depression in the blastogenic response of lymphocytes, and changes in the percentage of lymphocytes and subpopulations of lymphocytes. Of interest is the fact that many of these inhibitory effects can occur even in the presence of low concentrations of hormones (3). What is not yet clearly understood is what the impact is of
the release of endocrines vis-à-vis different kinds of stressors, for example, chronic stress versus acute stress, and what impact these changes have on the immune response, short term and long term.

As already discussed, physiological changes associated with stress include increases in blood levels of certain endocrines such as cortisol. Of interest and relevant to this discussion is a report by Markham et al. (42), who found that the ability to infect fresh normal human PBLs with HTLV-III productively was improved by supplementing cell culture medium with either the gonadal steroid chorionic gonadotropin or insulin, and even more substantially with the adrenal cortical steroid hydrocortisone. The data suggest a role for corticosteroids in the modulation of HTLV-III expression and/or replication and, put together with the data from our laboratory and others showing similar changes associated with stress, imply that there may be a connection. The high-risk populations for acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC), such as homosexual men, may be a more psychologically stressed group because of societal and sociological pressures. Furthermore, this population, which is aware of the AIDS association with homosexuality, is under additional psychological stress because of the knowledge of the association of this devastating illness with their sexual practices.

THE ACQUIRED IMMUNE DEFICIENCY SYNDROME

Over the past several years, a severe disease syndrome involving opportunistic infections and Kaposi sarcoma has become a major health concern in the United States and several other countries. The AIDS has been linked to a human T-lymphotropic retrovirus, referred to as HTLV-III, lymphadenopathy-associated virus (LAV) (HTLV-III/LAV) (13). Associated with this immunosuppressive illness are opportunistic infections, for example, *Pneumocystis carinii*, *Toxoplasma gondii*, bacterial infections, and viral infections such as HSV. A common denominator for these clinical entities is abnormalities in cellular immunity, particularly demonstrated by a reduction in helper (inducer) T lymphocytes and abnormalities in the helper T-cell to suppressor T-cell ratio, as well as other immune dysfunctions. In addition, complications involving the CNS have also been described (55). The isolation of HTLV-III/LAV has been accomplished from lymphocytes from both AIDS and ARC patients. It is not known what controls the clinical course of ARC patients to frank AIDS patients. As discussed in this chapter, it is possible that stress-associated immune depression may be one component that affects the clinical course of both ARC and AIDS.

IMPLICATIONS OF NEUROIMMUNOMODULATION FOR ARC AND AIDS PATIENTS

As already discussed, there is evidence that supports the theory that the etiological agent for immune dysfunctions culminating in clinically defined ARC and AIDS is related to the HTLV-III/LAV virus (4,13). Evidence for this association is
based primarily on a high correlation between the presence of antibody to viral structural proteins in the serum from a high proportion of patients as well as clinically healthy individuals with an elevated risk of being exposed, such as promiscuous homosexual males, heterosexual contacts of AIDS and ARC patients, and hemophiliacs (30,53). In addition, the isolation of HTLV-III/LAV from cultured lymphocytes from both ARC and AIDS patients as well as donors at risk for AIDS also supports the etiology of this virus(es) with the disease. It has also been shown that HTLV-III/LAV is cytopathic for T lymphocytes with the helper-inducer phenotype OKT-4/Leu-3+ (13). It is of interest and relevant to this discussion that HTLV-III/LAV virus was isolated from lymphocytes of nearly 80% of ARC patients but initially only from 35% of patients with AIDS (13,48). Therefore, simply having the virus and carrying the virus in T lymphocytes does not necessarily imply that a person will have clinical disease. For reasons that are not clear, only a certain percentage of ARC patients potentially become AIDS patients. What determines the clinical course of the disease is not yet known. However, it is possible that any negative modulation of the immune response could result in an impact on the relationship between the virus and the lymphocytes such that virus could be reactivated from “latently infected” T lymphocytes. This reactivated virus could then be available for infecting other T lymphocytes, and so on. If this happens concomitantly in an individual whose immune system is less efficient than normal, then enhancement of virus replication could take place.

**IMPLICATIONS FOR HTLV-III/LAV LATENCY AND PSYCHOLOGICAL STRESS**

The data obtained in studies from our laboratory, already discussed in this chapter, show that at least for three latent herpesviruses, psychological stress can impact on the ability of the cellular immune response to control the maintenance of the virus genomes in the cells in which the virus is latent. Are there circumstances that provide a similar situation for HTLV-III/LAV, and, if so, can we extrapolate from the data on herpesvirus latency to AIDS? We have shown not only that the EBV genome can be induced from cell lines in which the virus is latently associated with drugs such as IUDR but, more importantly, that a similar phenomenon actually takes place using NPC tumor biopsies.

In a recent study, Folks and co-workers (12) infected a human T-cell line, A3.01, with HTLV-III/LAV. The virus lytically replicated in these cells and killed a high percentage of the cells. However, a small number of cells that lacked the Leu-3 surface marker survived infection. After growing out these remaining viable cells and reestablishing the cell culture, they found that the cells did not produce virus and that they could not be infected by the virus as well. What is of interest, however, is that HTLV-III/LAV could be induced to replicate and produce infectious virus from the A3.01 cells after treatment with IUDR, even after long-term culture up to 3 months. It is possible that individuals who harbor the virus in the latent form may do so for significant periods of time in the absence of expression of virus
proteins or infectious viral particles, similar to cells latently infected with EBV. Under certain conditions, such as found for herpesviruses, it is possible that modulation of the cellular immune response and other factors involved in the reactivation of herpesviruses, such as stress, could result in the reactivation of the HTLV-III/LAV, perhaps as a consequence of the combination of down-regulation of cellular immunity and increases in stress-associated hormone levels. It is possible that this sequence of events could take place in clinically normal or ARC patients, resulting in a more acute form of immunosuppression, i.e., AIDS.

CONCLUSION

In this chapter, we have attempted to evaluate the data obtained in our laboratory and others on the psychological mediation of the immune response, sometimes called "psychoneuroimmunology" or "neuroimmunomodulation." We have demonstrated significant changes in several aspects of cellular immunity, many of which have potential significance for AIDS, such as NK cell activity and numbers and γ IFN production. We have also shown that reactivation of latent herpesvirus genomes may also be affected by psychological stress, presumably through the mediation of cellular immunity and its control over virus expression. The mechanisms whereby the nervous system and immune system react with each other are still not clear, but it is thought in part at least to be associated with certain endocrines. We have tried to speculate on how these phenomena may be related to AIDS or ARC. The data obtained in other studies on the expression of HTLV-III/LAV and hormones, and on the possibility that this virus can latently infect certain T lymphocytes and be reactivated with certain drugs such as IUdR, may have implications in regard to the data obtained in our laboratory on herpesviruses. Whether psychological stress in some way can modulate the expression of HTLV-III/LAV and whether factors such as stress can have implications for whether an ARC patient progresses to AIDS and the rate of appearance and severity of clinical symptoms remain speculative, and further studies will be necessary to determine whether this is so.

REFERENCES


