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## Stress-Associated Immune Modulation and Its Implications for Reactivation of Latent Herpesviruses

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### I. INTRODUCTION

There is an extensive literature concerned with the integration of the immune system with the central nervous system (CNS) and endocrine systems. Each of these body systems is complex, and studying the interactions among the three systems raises the complexity by orders of magnitude. The field of “psychoneuroimmunology” or “neuroimmunomodulation” is a rapidly developing field and encompasses these interactions; it also includes the study of behavior as a modulator of the interaction.

There is experimental evidence suggesting that the CNS/immune axis is bidirectional. The immune system receives signals from the CNS (e.g., neuropeptides) and from the endocrine system (e.g., glucocorticoids, prolactin, and growth hormone). Feedback from the immune system modulates the CNS—for example, the influence of interleukin-1 (IL-1) on the hypothalamus. More than likely, this feedback also includes other cytokines and growth factors made by lymphocytes themselves, such as the adrenocorticotrophic hormone (ACTH) (1). It is clear that one needs to think about the historical roles of lymphokines, growth factors, hormones, and neuropeptides in an expanded way to include a much broader biological responses.

Two central issues still to be addressed are (1) what are the mechanisms whereby the CATS, endocrine, and immune systems interact with each other and (2) what are the health implications of these interactions? This chapter attempts to address the second question with the proviso that the long-term longitudinal studies needed to explore these interactions are still under way. However, there is ever-increasing evidence that the interactions among these systems could have implications for illness, particularly in regard to herpesviruses.

It has now been well established that a variety of stressors can promote the release of pituitary and adrenal hormones. For example, psychological stress in humans increases the levels of corticotropin-releasing factor (CRF) via the hypothalamus, which then stimulates the pituitary gland to produce ACTH, which then stimulates the adrenal gland (the HPA axis), modulating a variety of immune interactions (2). Studies on the relationship between neuroendocrine peptides and modulation of immune function have focused on neuropeptides derived from the polypeptide pro-opiomelanocortin (POMC), particularly ACTH and beta endorphin. However, as already mentioned, other hormones—such as cortisol, growth hormone, prolactin, the catecholamines, epinephrine, and norepinephrine—have also been shown to modulate immune function (1).

There is a large and consistent literature on stressful life events that suggests that individuals who have experienced a recent major negative life change may be at greater risk for a variety of illnesses, including infectious disease. While the correlations are not large, generally explaining about 10% of the variance (3), the effects are remarkably consistent. Of interest is the fact that certain events, those associated with the loss of important personal relationships such as bereavement or divorce / separation, clearly put individuals at greater risk for illness. Social support modulates morbidity and mortality, accounting for as much of the variance for risk for illness as smoking, exercise, and being overweight (4). Many of these issues have been the focus of studies in psychoneuroimmunology. The immunological and pathological consequences of bereavement, one of the most stressful life events, has been the subject of several studies. There is, for example, evidence that the widowed survivor experiences an increased risk of morbidity and mortality following the death of the spouse (5,6). In some of these studies, immune impairments have been described associated with this loss, suggesting that immune competence may play a role for the health risk in these individuals (7-11). As already indicated, divorce is also associated with immunological alterations, particularly in individuals who have been separated for only a short period of time and who are still strongly attached to their spouse (7).

One of the key issues in the field of psychoneuroimmunology has been the connection between stress-related immunological alterations and actual health changes. While it is reasonable to assume that both long- and short term alterations in immune function have deleterious consequences for health, there are a few studies that provide evidence of such relationship (1,8-14). There are several problems in establishing these relationships; for example, stressed or distressed individuals are likely to have a variety of life style factors that would put them at a greater risk independent of the immune system interactions. These include poor health habits such as alcohol and drug abuse, poor sleep, poor nutrition, less exercise, etc. (14). In addition, persons who are socially isolated, who have demonstrably higher morbidity and mortality (4), are also less likely to have contact with others and in that regard may be less likely to be infected with a pathogen.

## II. THE IMPACT OF ACADEMIC STRESS ON THE IMMUNE SYSTEM

In studies from our laboratory, we were interested in determining if a relatively commonplace stressful event could be associated with suppression of the immune response, and if the suppression could have implications for risk for infectious disease, such as upper respiratory tract infections. In order to explore this possibility, we have performed several studies which involved first- and second-year medical students at The Ohio State University Medical Center, using academic stress as an acute cyclical stressor. The curriculum in the first 2 years at The Ohio State University College of Medicine is such that the medical students do not take examinations at random; the examinations occur in 2- to 3-day examination blocks (approximately seven or eight of these), across the academic year. Therefore, the medical students, as a class, cycle together in either less stressful periods or more stressful periods in which they are taking examinations.

In our first study, we found evidence for a decrease in natural killer (NK) cell activity (using K562 cells as target cells) in blood samples obtained from 75 medical students during their examination periods, in contrast to blood samples obtained at the baseline period 1 month before. We also found that psychological distress increased during examination periods as compared to the baseline period (15). Natural killer cell activity is thought to be important as a defense not only against cancer but also against virus infections (16). In a subsequent study, we confirmed these results with NK cell activity using a different target cell, MOLT-4. The decrease in cell killing was accompanied by decreases in the number of large granular lymphocytes, the

NK-cell phenotype, and the number of cells detected with the NK monoclonal antibody (MAb) Leu-7 (17). These data suggest that the percentage of NK cells and their ability to kill two different target cells could be modulated by psychological stress.

Other changes in the cellular immune response were also negatively associated with academic stress. These include the percentage of total T lymphocytes, changes in the percentages of helper and suppressor T lymphocytes (18), and the ability of peripheral blood leukocytes (PBLs) to proliferate in response to the T-cell mitogens, concanavalin-A (Con-A) and phytohemagglutinin (PHA) (8,18). In addition, we have found that PBLs obtained from the medical students during examination periods showed a lowered ability to produce gamma interferon in response to stimulation with Con-A, and PBLs from the medical students showed a significant increase in intracellular levels of cyclic AMP (cAMP) as well as an increase in plasma levels of CAMP during examination stress (19). These data are consistent with the down regulation of cell function observed across studies.

In these studies, it was important to rule out other factors that could have been confounds in interpreting the data linking the association of the immune changes observed with psychological stress. For example, it has been shown that nutrition can have a significant impact on the immune response (20). In order to rule out the possibility that the medical students (and other subjects studied in other studies in our laboratory) were not showing changes related to nutrition, plasma levels of albumin and transferrin were obtained. Any person who did not fall within the normal range was excluded from the study; it was rare that a person fell outside normal ranges of these nutrition markers. In addition, data were obtained on alcohol intake, weight loss or gain, sleep patterns, cigarette smoking, caffeine intake, and medication use, particularly immunoreactive drugs. None of these data correlated with the immune changes that were observed in studies with the medical students or with other subjects in other studies.

### III. THE CELLULAR IMMUNE RESPONSE: IMPLICATIONS FOR LATENT HERPESVIRUS INFECTIONS

When a person is infected with one of the human herpesviruses, seroconversion often results in the absence of clinical disease. Whether a person becomes clinically ill after virus infection, however, apparently does not have impact on the ability of the herpesvirus to persist in a latent state, presumably for the lifetime of that individual. Under certain conditions, any one of the herpesviruses can be reactivated to lytically replicate. However, neither the

mechanisms underlying the establishment of latent virus infection in an appropriate target cell nor the mechanisms underlying viral reactivation are well understood. What is known, however, is that virus reactivation can take place in the presence of high levels of circulating antibody. The cellular immune response plays a very important role in the maintenance and replication of latent herpesviruses and presumably, the reestablishment of control over the virus after reactivation.

In a normal individual who has a "normal" cellular immune response, the reactivation of latent herpesviruses can occur sporadically and often does so in the absence of clinical disease. Reactivation is usually accompanied by a noticeable increase in specific antibody titer to the virus, even in the absence of detectable infectious virus. However, in extreme cases where a person is severely immune-suppressed, as after renal transplantation, reactivation of one or more of the latent herpesviruses can result in severe morbidity and mortality (21-26).

It is also possible to find individuals who demonstrate prolonged virus shedding not associated with disease, such as persistent asymptomatic shedding of cytomegalovirus (CMV) in the urine or Epstein-Barr virus (EBV) in the saliva of normal individuals (27,28). The appearance of infectious herpes simplex virus (HSV) in the saliva or vaginal secretions in the absence of lesions can also be observed periodically (29,30). It is generally accepted that the cellular immune response, as opposed to neutralizing antibody, plays an extremely important role in the maintenance of virus latency, and that this conclusion is supported by the fact that recurring HSV-associated disease can occur in the presence of high levels of neutralizing antibody, as already discussed (31-33). In the case of HSV, severe impaired cellular immunity can be associated with both mild and severe clinical disease (34) as well as recurring disease (35,36).

Studies on renal allograft recipients have shown that immune suppression can lead to the impairment of an EBV-specific memory T-cell response and increases in anti-EBV antibody titers (21). In addition, renal allograft and other organ transplant recipients show a higher incidence of virus shedding than normal controls under long-term immunosuppressive therapy (37).

It is known that the efficiency of immune function decreases with aging, the impact of this change can be found, for example, in the incidence of herpes zoster virus, which increases with age, as does the severity of illness (38,39). In addition, herpes zoster is more prevalent in patients who have malignant disease and in patients receiving radiation therapy or immunosuppressive agents compared to normal individuals (40). When EBV antibody titers in a healthy geriatric population were studied, antibody titers to EBV

**Table 1** Geometric mean titers of antibody to EBV antigens in a young adult population and a geriatric population

Population	EBV Antibody		
	EA (IgG)	VCA (IgG)	VCA (IgA)
Geriatric	459	662	17
Students (young adult)	53	94	3.6

Source, From Ref. 41

early antigen (EA) and virus capsid antigen (VCA) IgG and IgA were higher in older individuals than EBV titers in a younger group of individuals, -i.e., medical students (41) (Table 1). A similar age effect has been observed in individuals who are antibody-positive for CMV (24).

#### IV. STRESS-ASSOCIATED CHANGES IN THE CELLULAR IMMUNE RESPONSE: IMPLICATIONS FOR LATENT EPSTEIN-BARR VIRUS INFECTIONS

Among the various factors thought to be associated with the reactivation of latent herpesviruses is psychological stress. Considerable anecdotal speculation has linked stress and the appearance, duration, and intensity of herpes virus infections, presumably as a result of the modulation in the cellular immune response already discussed. Psychological stress has been shown to contribute to the development and severity of EBV-associated infectious mononucleosis (IM) (42), Katcher and coworkers (43), Friedmann et al. (44), and Goldmeier and Johnson (45) have shown that stress can be associated with reactivation of oral and genital herpesviruses. In an early study on the relationship between stress and viral infections, Rasmussen et al. (46) demonstrated that stressed mice are more susceptible to infection with HSV as compared to the control group. Recent studies by our laboratory confirmed and extended these studies (47,48). These data will be discussed in another section. In a study of Marek's disease herpesvirus (MDHV) in chickens, chickens introduced into a new pecking order had a higher incidence of MDHV (49). The MDHV causes malignant and neurological disease in chickens.

Using the academic stress model with medical students at The Ohio State University College of Medicine, as already discussed, we have explored the

possibility that psychological stress could play a role in the reactivation of latent EBV and HSV. The studies were performed by measuring antibody titers to both viruses over time, both at baseline periods in which the medical students were confirmed to be less stressed and during examination periods when the medical students were more stressed (19,50). In these studies, we found reliable changes in antibody titers to these two viruses concomitant with the down-regulation of different measures of the cellular immune response. As already discussed, elevated antibody titers to herpesviruses are thought to reflect the increased production of viral antigens after reactivation that have subsequently induced a memory response, resulting in an increase in the production of antibodies. In the first study (50), we found more than sixfold higher antibody titers to EBV VCA IgG—using the indirect immunofluorescence (IF) test—during final examinations in first-year medical students compared to titers after the same students returned from summer vacation in September. In addition to the higher EBV VCA antibody titers, higher antibody titers to HSV type 1 and CMV were found during final examinations as compared to antibody titers after summer vacation. In order to explore the possibility that the changes in antibody titers to EBV, HSV, and CMV were associated with a general polyclonal activation followed by a non specific rise in total IgG, we measured antibody titers to poliovirus type 2 in the same plasma samples; we found no significant changes in the poliovirus antibody titers.

In a follow-up study we studied medical students across the entire academic year, i.e., three lower-stress "baseline" periods and three higher-stress examination periods. Antibody titers to EBV VCA were reliably elevated during examinations (19) (Table 2). In the same study, we found that the ability of EBV-specific memory (cytotoxic) T-lymphocytes to inhibit the outgrowth of EBV-infected autologous B lymphocytes (using the 50% regression assay) also changed across the academic year, with less cell killing observed at the time of examinations as compared to the baseline periods (Table 2). The regression assay is thought to measure specific EBV T-cell killing. Harada and colleagues (51) have shown that immune-suppressed x-linked lymphoproliferative disease patients (XLP) show a significant decrease in the cytotoxic T-cell response to autologous EBV-infected B cells using this procedure. These data were interpreted as an impaired lymphocyte response to EBV-specific antigens and a defect in memory or helper T-cell function.

Since we found changes in HSV-1 specific antibody levels associated with changes in psychological stress, we examined another aspect of HSV reactivation by studying a factor designated leukocyte migration inhibition factor (LIF) (36). The LIF is a protein that inhibits the migration of polymorpho-

**Table 2** Means ( $\pm$  SEM) for EBV VCA Antibody Titers and EBV I

Sample	EBV VCA <sup>a</sup>	EBV I
Baseline 1 (September)	68.18 (25.05)	NT <sup>b</sup>
Examinations (October)	115.04 (10.86)	NT
Baseline 2 (January)	80.08 (15.86)	8.75
Examinations (February)	386.36 (101.30)	3.68
Baseline 3 (April)	84.85 (22.76)	11.01
Examinations (May)	141.03 (46.34)	4.11
	$n = 34$	$n = 2$
	$p = <.0001$	$p = <$

*Source.* Adapted from Ref. 19. <sup>a</sup>Determined by indirect IF <sup>b</sup>NT = not

nuclear leukocytes but not monocytes. It has been previously shown that the ability of PBLs from HSV-2-seropositive individuals to produce LIF in vitro in response to HSV-2 specific stimulation is suppressed during recrudescence of HSV-2 infections as compared to cells obtained from individuals during the convalescent phase of HSV infections. In a follow-up study, when LIF was assayed in this group of medical students, an inhibition at the time of examinations was observed. These data suggest that at least three different markers associated with two latent herpesviruses (antibody titers, the memory T-cell response to EBV, and HSV-specific LIF production) were modulated in the same individuals by academic stress.

In a subsequent study, we explored whether it was possible to detect EBV in the nasopharynx at the same time that antibody changes to the virus were observed. It has been shown that the utilization of DNA dot-blot hybridization, probing EBV DNA extracted from exfoliated cells obtained from throat washing samples from individuals who are EBV-seropositive, is possible; this approach allowed the detection of reactivated EBV, providing a reliable measure of the presence of infectious virus which is commonly detected by measuring transforming activity (associated with infectious EBV) of throat washing preparations (52).

When we attempted to measure EBV DNA in exfoliated cells obtained at three baseline and three examination periods, EBV DNA was detected in only one specimen of one subject (at the time of examinations). Thus, in this group of medical students, while antibody changes were being observed, it was not possible to detect any significant release of infectious EBV, at least by dot-blot hybridization.



We hypothesized that it might be possible to explain changes in antibody levels (in the absence of the ability to detect infectious virus) by partial reactivation of the virus genome. If this were the case, the increase in antibody titers observed by the indirect IF test could be due to the expression of only certain viral genes. In that event, the EBV proteins encoded by these genes could induce the appropriate memory immune response, more or less. However, the use of the indirect IF test using plasma or serum would not have permitted us to discriminate between complete and incomplete virus reactivation, since we would be measuring essentially all EBV antibodies present in the samples.

In order to test this hypothesis, MAbs to four EBV polypeptides (two early and two late) were measured by an ELISA. These viral polypeptides were obtained using EBV-specific MAbs as described by Pearson and coworkers (53). Plastic plates coated with each polypeptide were prepared and used to retest the same plasma samples that had been used in earlier studies in which stress-associated fluctuations of EBV antibody (by IF) had been found (Fig. 1). By using this procedure, it was possible to determine whether there were any differences in the levels of a specific antibody to one specific EBV polypeptide in each sample.

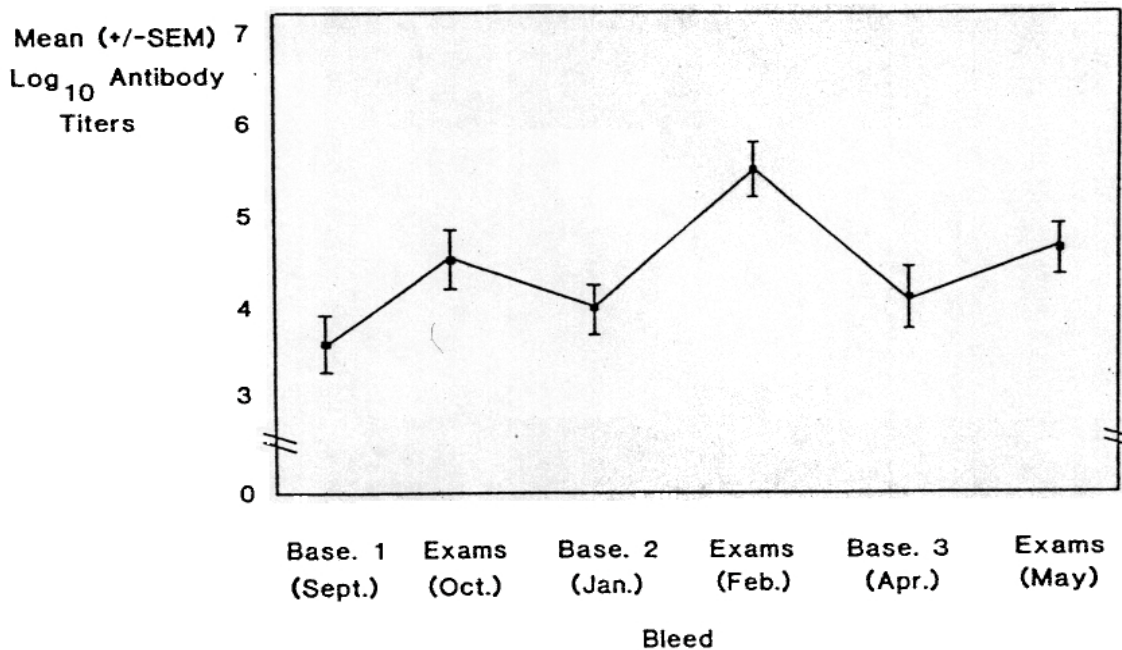


Figure 1 Antibody titer to the EBV VCA complex of proteins (IgG) across three baseline and three examination blood samples. Antibody titers were determined using the indirect IF test. (From Ref. 54.)

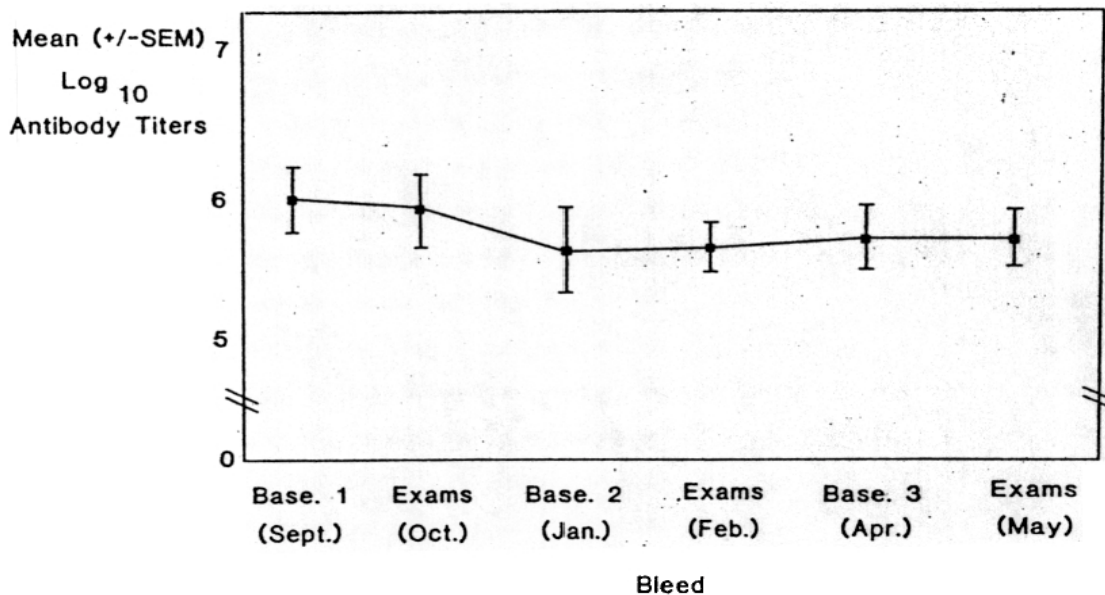


Figure 2 Antibody titers to the 125-kDa EBV VCA protein across three baseline and three examination blood samples. Antibody titers were determined using an ELISA. (From Ref. 54.)

The data obtained in this study suggest that incomplete reactivation of EBV did occur in these medical students over the academic year (54). Supporting this interpretation were data showing that plasma samples with higher VCA IgG antibody titers at the time of examinations, as measured by IF, showed no changes in antibody titers to two late viral proteins, the 125-kDa VCA polypeptide and the Gp 350/300 membrane antigen (MA) (Figs. 2 and 3). Similarly, when the 85-kDa early antigen (EA-R) protein was used, again no evidence for changes in antibody titers was observed (Fig. 4). However, antibody titers to the early EA diffuse (EA-D) 52/50-kDa protein were modulated over the course of the study (Table 3). To our surprise, we found that there were individuals who did not have a detectable (< 1:2) antibody titer to the 52/50-kDa EA-D polypeptide in an early blood sample but became antibody-positive in a later blood sample and some individuals who were antibody-positive in an early blood sample but became antibody negative over the course of the study. It is possible that the expression of the gene encoding for this protein was down-regulated sufficiently to cease the production of this protein or the down-regulation of the production of the protein was sufficient to reduce the amount of antigen available to stimulate antibody production.

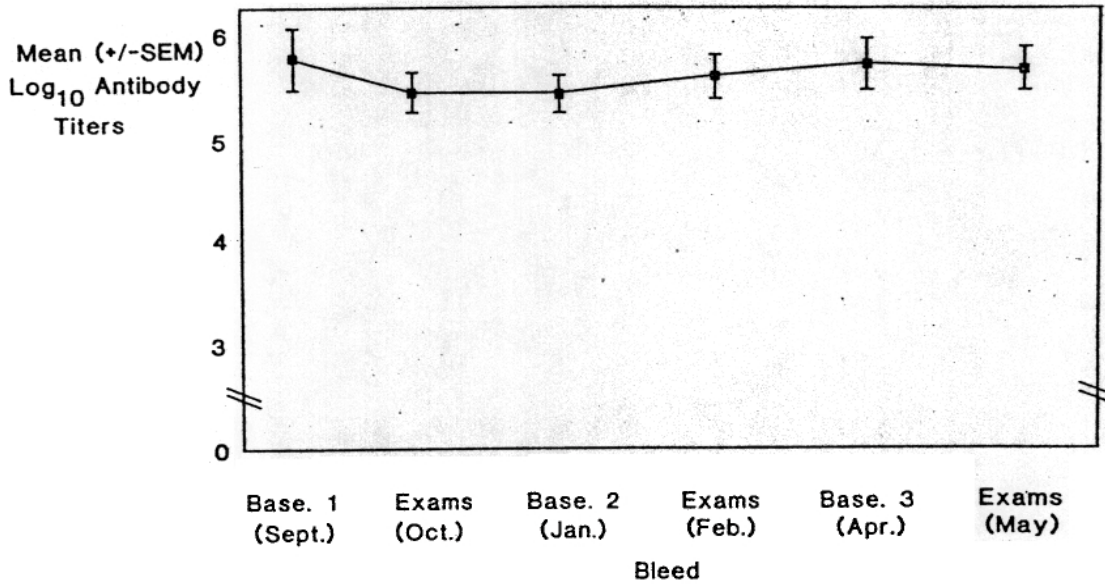


Figure 3 Antibody titers to the late EBV MA, GP 350/300, across three baseline and three examination blood samples. Antibody titers were determined using an ELISA. (From Ref. 54.)

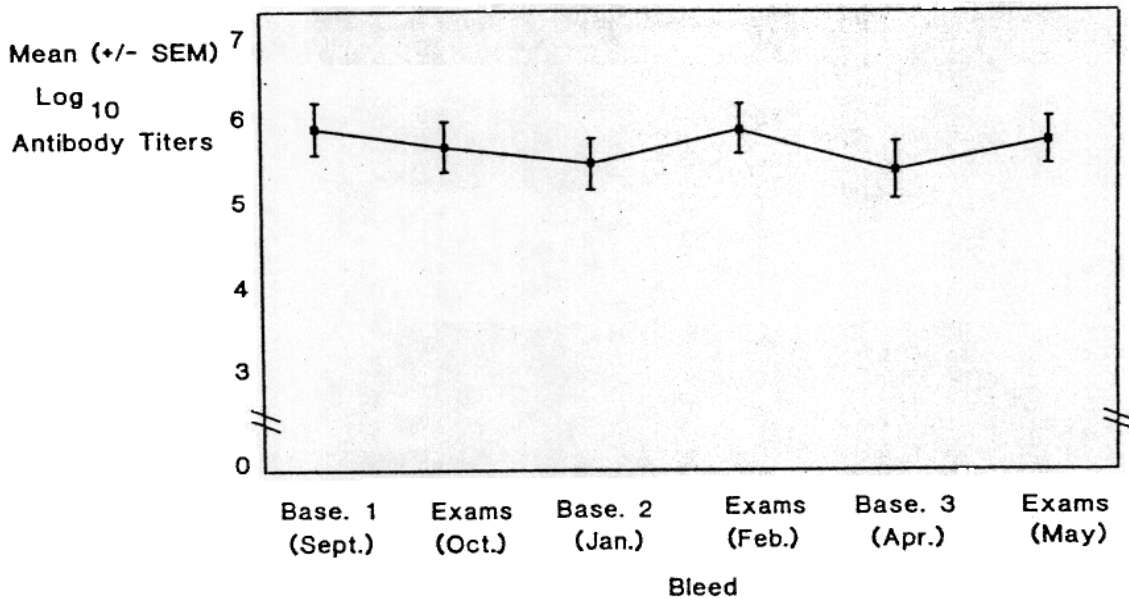


Figure 4 Antibody titers to the EBV 85-kDa EA-R protein across three baseline and three examination blood samples. Antibody titers were determined using an ELISA. (From Ref. 54.)

Table 3 Percentage of subjects with antibody to the 52/50-kDa EA-D EBV protein at each sample point and mean antibody titers ( $\pm$  SEM) of those who were antibody-positive

Bleed	N	No. Positive	% Positive	Antibody Titer: (log <sub>50</sub> )	
				Mean	$\pm$ SEI
Baseline (September)	15	6	40.0	1.11	.38
Exam (October)	15	13	86.7	3.19	.49
Baseline (January)	15	9	60.0	3.47	.81
Exam. (February)	15	6	40.0	1.20	.41
Baseline (April)	15	0	0.0	.00	.00
Exam (May)	15	2	13.3	.37	.25

Source. From Ref. 54.

Note: Cochran's test;  $Q = 30.0$ ;  $p < .001$

When the antibody pattern to the EBV 52/50-kDa EA-D protein was compared to the data obtained by IF, we found that the pattern was not the same between the two studies. It is possible, therefore, that other proteins may be modulated in a selective way, and that antibodies to these proteins could be synthesized at different time points and detected by IF. Additional EBV specific polypeptides need to be used to extend these studies.

Finally, in a recent study we investigated the memory T-cell (proliferative) response to several early and late EBV polypeptides. Blood samples were collected twice, 1 month before a 3-day examination block and again on the last day of the examination series. Subjects were 25 healthy EBV-seropositive medical students. The following purified EBV polypeptides were used: gp 250, gp 125, p 160, p 85, p 52/50, and p 17. These polypeptides were prepared from HR-1 cells (a virus-producing Burkitt lymphoma [BL] cell line) expressing the different EBV antigens. Using cell-free supernatants sequentially passed over affinity columns prepared with MAbs to the respective polypeptides, significant decreases in T-lymphocyte proliferation were found in response to gp 250, p 160, p 17, p 52/50, p 85 during examinations compared to baseline. In addition, there was a marginally significant decrease in the proliferative response to gp 125 (55). As already discussed, we had demonstrated that there was a decrease in the EBV-specific memory (cytotoxic) T-cell response modulated by academic stress using the medical student model (19). These data provide further evidence that psychological stress can modulate the memory cellular immune response to latent EBV.

## V PSYCHOSOCIAL ISSUES, IMMUNE FUNCTION, AND EPSTEIN-BARR VIRUS LATENCY: HEALTH IMPLICATIONS

There is excellent evidence linking interpersonal relationships and health (4). These studies have shown that there is greater mortality among individuals with fewer or less satisfying close relationships. Although this association is now well established, the mechanisms underlying this association is yet to be understood. Levy and coworkers (56) found a connection between immune function and social support in patients with breast cancer. The study explored the relationship of NK cell activity and psychological status at base line and 3 months into the treatment of breast cancer patients. Social support accounted for 30% of the variance in NK-cell activity at the 3-month follow up. Their data controlled for the effect of radiation and chemotherapy on NK cell activity.

In two studies from our laboratory, we found that lonelier medical students (i.e., those medical students scoring above the median on a test which measures loneliness (the UCLA loneliness scale) had significantly lower levels of NK-cell activity than students who described themselves as less lonely (15). The same group of medical students also had higher antibody titers to EBV VCA IgG (50).

In another study, lonelier psychiatric patients showed lower levels of NK cell activity than less lonely patients as well as a poorer T-cell proliferative response to PHA and higher levels of stress-related urinary cortisol (57). We have continued to explore the relationship between social support and immune status. In two cross-sectional studies involving marital quality and immune status, we found that poorer marital quality in 38 women was correlated with higher EBV VCA IgG antibody titers as well as poorer blastogenic responsiveness of their PBLs to PHA and Con-A (7). Similar results were obtained in a sample of married men (58); those men who described their marriages as poorer had higher antibody titers to EBV VCA IgG and lower Thelper/suppressor cell ratios. Thus, taken together, data from these two studies are consistent with previous reports linking the quality of marital relationships with changes in certain aspects of the immune response, mental, and physical health (59).

A study using West Point cadets was performed by Kasl and coworkers (42). They followed West Point cadets who were EBV-seronegative upon entry to the West Point Military Academy over the 4 years, assessing seroconversion to EBV, clinical symptoms of IM, and severity of illness. These clinical data were studied in regard to a triad of psychosocial risk factors: poor academic performance, higher levels of motivation for a military career, and having a father who was an "overachiever"; this triad was associated

with greater risk for seroconversion to EBV, longer hospitalization in the infirmary following seroconversion, and elevated EBV VCA antibody titers among those who did seroconvert but who had no clinical symptoms.

In other studies, a number of investigators have compared herpesvirus antibody titers in psychiatric patients with those of control subjects in order to try to identify one of these viruses as the etiological agent for the psychiatric illness. Depressed psychiatric patient subgroups have had significantly higher herpesvirus antibody titers than nonpsychiatric controls. When antibody titers to other viruses, such as measles or rubella virus, have been studied, antibody titers to these viruses have not been found to be elevated (60). These observations have led some researchers to speculate on the possible etiological significance of herpesvirus infections for certain clinical disorders. However, given the stress-associated changes in antibody titers to EBV, HSV, and CMV observed in our studies, it is possible that a more parsimonious explanation for these herpesviruses antibody titers may be related to the greater distress of psychiatric patients as compared to controls, rather than an etiological relationship.

The EBV has also been associated with depression (61-63) and with chronic or chronic fatigue syndrome (CFS). There is some evidence that psychopathology or stress may be part of the sequence of events that take place prior to or during the course of CFS, and it has been postulated that a neuroimmune interaction may be a "cofactor" (64-66). Consistent with this hypothesis is the fact that several of the immunological findings described in patients with CFS (66,67) include elevated antibodies to EBV, an inhibition of the response of PBLs to mitogens, a decrease in NK-cell activity, and a decrease in EBV-specific cytotoxic T-cell activity. As already discussed in this chapter, all of these responses have also been associated with psychological stress.

Several studies by Esterling and coworkers have confirmed that psychosocial modulation of latent EBV can occur in different groups of individuals. The data provide evidence for reactivation of EBV as measured by increases in EBV VCA associated with emotional repression in 80 first-year undergraduate students (68). In a more recent study, the same group examined the effects of two behavioral interventions, exercise training and cognitive behavioral stress management, in gay men at risk for infection with HIV. They found that these interventions modulated the expression of latent EBV as demonstrated by a decrease in antibody titers to EBV VCA and human herpesvirus type 6 in HIVA infected and at risk gay men (69). Antibody titers to EBV VCA were also found to be associated with differences in repressive coping styles in a group of 54 healthy young college students (70). Still another study examined how cognitive changes in experimental involve-

ment during an emotional disclosure induction protocol can modulate cellular immunity and latent EBV VCA as measured by antibody titers in 76 college undergraduate students (71). Thus the pattern emerging from work from our laboratory and others suggests that immune modulation associated with behavior (such as psychological stress) affects the ability to control latent EBV and other latent herpesviruses.

While not related to herpesviruses, two recent studies provide solid evidence that stress has clear relevance for infectious illness. Cohen et al. prospectively studied the relationship between stress and susceptibility to colds by inoculating volunteers with 5 different cold viruses or a placebo (14). They found that rates of both respiratory infection and clinical symptoms (colds) increased in a dose-response manner with an increase in psychological stress and that this was true for all five strains of respiratory viruses. These data provide an outstanding, well-controlled demonstration of increased infection to viruses associated with increased stress (14).

Consistent with Cohen et al., a recent study from our laboratory showed that stress can influence the way medical students respond to a viral vaccine. We gave each of a series of three recombinant hepatitis B (Hep B) inoculations to 48 medical students on the last day of a 3-day examination series in order to study the impact of academic stress on the ability of the students to generate an immune response to a primary antigen. We found that one quarter of the medical students seroconverted after the first injection and that this same group of medical students were significantly less stressed and less anxious than those students who did not seroconvert until after the second injection. In addition, students who reported greater social support demonstrated a stronger immune response to the vaccine at the time of their inoculation as measured by antibody titers to Hep B surface antigen and the T-cell response to a purified Hep B viral peptide. We had followed this group of students for 1<sup>1</sup>/<sub>2</sub> years prior to this vaccine study, and the early and late seroconverters had not differed on anxiety, perceived stress, or social support prior to the first injection. These data suggest that the immunological response to a primary antigen, in this case the Hep B vaccine, can be modulated by a relatively mild stressor in young, healthy adults—a finding that may have public health implications (12).

## VI. STUDIES ON THE EFFECT OF STRESS ON HERPES SIMPLEX VIRUS: AN ANIMAL MODEL

Herpes simplex virus in humans is characterized by its ability to induce an acute infection and then establish a latent infection in local sensory ganglia which innervate the site of initial infection (72). Characteristic HSV infection is its ability to spontaneously reactivate from a latent, noninfectious state

to induce recurrent lesions at the periphery or near the site of infection (73). It is generally accepted that the level or degree of immune competence of an infected individual has impact on the rate of occurrence of reactivation as well as the severity of recurrent episodes. As briefly discussed earlier, the immune response to HSV infection (and other herpesviruses as well) is complex and involves components of both the humoral and cellular immune systems. However, it is the cellular immune response that is believed to play a more significant role in control of latent HSV infection and recrudescence. It is known that cell-mediated immunity plays an important role in controlling severe recurrent herpetic infections (34,74), as well as the history of recurrent disease (35). The fact that HSV can be transferred from cell to cell without entering extracellular spaces suggests that cell-mediated immunity plays a very important role in reducing clinical lesions by limiting the spread of infectious virus and destroying virus-infected cells. The HSV-specific cytotoxic T-lymphocytes (CTLs) presumably play an important role in limiting and controlling severity of recurrent infections (75).

As already discussed, psychological stress can affect the cellular immune response across a variety of immune functions. However, little is known of the effect of these interactions on the development of specific antiviral immune responses. Our laboratory has utilized an established murine model of an acute local footpad HSV type 1 infection in order to explore the effect of stress (restraint stress) on the generation of HSV-specific CTLs and HSV-specific memory CTL (CTLm).

In the design of such experiments, it was important to keep in mind that infectious HSV is a "replicating antigen." Previous animal studies on stress and immune function have primarily focused on immune response to "nonreplicating antigens," such as keyhole limpet hemocyanin, lipopolysaccharide, and sheep red blood cells (47). This is an important point, because non-replicating antigens are quickly cleared from the circulation by various components of the reticuloendothelial system, and, unlike infectious virus, do not provide a continuous source of antigenic challenge to lymphocytes in the spleen and lymph nodes.

In these studies, mice were inoculated with HSV-1 in the footpad; then the animals were stressed by being restrained for various periods of time and for various numbers of days in order to explore the possibility that this type of stressor could modulate the specific immune response to HSV. Food- and water-deprived control animals were used as a comparison group. The lymphocyte lymphoproliferative responses and the generation of HSV-specific CTL in the popliteal lymph nodes following footpad infection were depressed in restrained mice as compared to the infected, unrestrained control animals. Additional experiments were performed to determine the frequency analyses



of HSV-specific pre-CTL lymphocytes. Suppression of the CTL response occurred early in the sequence of events that preceded the generation of functionally lytic CTLs, and this was not mediated by a diminished interleukin-2 (IL-2) response. Concomitant with this down-regulation of the generation of HSV-specific CTLs, there was an increase, as compared to the control animals, in the recovery of infectious HSV at the site of infection. These data provide additional evidence that physiological changes associated with restraint stress in mice can influence the immune response to an HSV infection and therefore potentially alter the course of viral pathogenesis, defined in these studies as the primary infection (47).

In a follow-up study, we explored the possibility that a similar stressor might have impact on the development of HSV-specific CTLm following local and systemic HSV infection, since the acquisition of immunological memory is an important aspect in the long-term defense against HSV infection. It is important to explore the possibility that this aspect of the immune response to HSV might be modulated by stress. The results showed that restraint stress did not inhibit the generation of HSV-specific CTLm. However, restraint stress did inhibit the ability to activate CTLm to kill the appropriate target cells. Thus, the second study shows that activation of HSV-specific immunological memory can also be inhibited by physiological changes associated with stress. These results suggest that this relationship with stress could be part of the mechanism for the development of recrudescence of herpetic disease (48).

Using the same murine model, we explored the role of the adrenal gland in the restraint stress-induced suppression of viral immunity. In this study using adrenalectomized animals, it was found that restraint stress-induced suppression of generalized lymphadenopathy associated with local HSV infection was not mediated by adrenal-dependent mechanisms. However, stress-induced suppression of the development of HSV-specific CTLs *was* under the influence of the adrenal gland. Corticosterone alone was not responsible for suppression of CTL development. In combination with some yet undefined stress-associated adrenal-independent event(s), corticosterone was able to suppress both HSV-specific CTL development and associated lymphadenopathy (7b).

## VII. POSSIBLE MECHANISMS OF REACTIVATION OF LATENT HERPESVIRUSES ASSOCIATED WITH PHYSIOLOGICAL CHANGES INDUCED BY STRESS

The complexity, not only within each of the three body systems under discussion but the interactions among these three systems, is great. Attempting, therefore, to understand the mechanisms whereby a variety of stressors can

modulate the immune response, with its potential health consequences, is an important area to be explored. There are, however, some possible explanations that can be proposed as a starting point in trying to understand these very interesting interactions.

It is known that latent EBV and other herpesviruses can be reactivated *in vitro* by hormones such as glucocorticoids. For example, hydrocortisone can induce latent EBV and enhance CMV replication *in vitro* (77-80). It has also been demonstrated that glucocorticoid hormones can enhance HIV replication *in vitro* as well (81). As already discussed, glucocorticoids, as well as other hormones, are released during psychological stress after the activation of the HPA axis (2). Therefore, it is possible that stress reactivates latent herpesviruses by directly inducing latent virus via increased levels of one or more stress-associated hormones or neuropeptides. Simultaneously, the down-regulation of the cellular immune response which is associated with stress could reduce the ability of the cellular immune response to control latent virus once reactivation has taken place. The end result would be consistent with observation from the human and animal studies outlined in this chapter.

In the study of medical students already discussed, we have evidence that partial reactivation of latent EBV can occur. The extensive literature demonstrates that it is possible to obtain incomplete or total reactivation of latent EBV *in vitro*, depending upon the cell line used and the treatment employed. When using the EBV genome-positive nonproducing BL tumor cell line Raji, only the EBV EA complex can be detected after treatment of the cells with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) or the halogenated pyrimidine 5'-iododeoxyuridine. When Raji cells are treated with these two drugs, EBV DNA synthesis does not occur, nor is there expression of late viral proteins, including VCA. Using other BL cell lines, such as HR-1, and epithelial hybrid cells that are EBV-genome-positive as well, one can study the induction of latent EBV under a variety of conditions in some detail. When these cells are treated with virus-inducing drugs, it is possible to induce EA, VCA, and virus particles (82-84). The data suggest that cellular mechanisms play a very important role in controlling EBV gene expression.

These data may have implications for clinical disease. Antibody titers to different components of the EBV EA complex, EA-R and EA-D, vary in patients with different EBV-associated diseases, including IM, BL, and nasopharyngeal carcinoma (NPC) (reviewed in Ref. 85). Furthermore, there are examples of EBV-associated diseases, such as CFS and NPC, in which antibodies to the EBV-induced enzymes DNA polymerase and DNase can be

detected and used in a clinical setting. Of interest is the fact that antibodies to these two enzymes are not detectable to any great extent in patients with IM or BL. These observations, when taken into account with the results of *in vitro* activation of EBV with TPA and MR, suggest that EBV gene regulation *in vivo* may not be an all-or-none phenomenon. This hypothesis is supported by the medical student study where a similar outcome was observed. It is possible that different gene products of the herpesviruses may be expressed under different conditions *in vivo*. If this is the case, this could result in different antibody patterns; in the case of EBV, this appears to have clinical relevance.

It is not clear how hormones such as glucocorticoids can induce latent EBV. However, in a recent study from our laboratory, we have explored another aspect of the impact of psychological stress on immune function that could be related to this question. We have studied the impact of psychological stress on radiation-induced apoptosis (cell suicide) in PBLs from the medical students. Apoptosis is the process of genetically programmed intracellular alterations that leads to the failure of proliferation and differentiation and eventually to cell death. Apoptosis may be induced by a variety of toxic insults, including growth factor deprivation and ionizing radiation. It has also been demonstrated that phorbol esters can inhibit radiation-induced apoptosis (86-89).

In our study, the PBLs were obtained during one of the studies on academic stress with the medical students at a baseline period, during an examination block, and then again at a baseline period following the examination block. Apoptosis was induced in the PBLs using low levels of radiation in the presence or absence of TPA. The results showed reversible changes in apoptosis in the PBLs in response to the low levels of irradiation and TPA, related to the stress of taking examinations (90). Taken in the context of the immune changes already discussed, it adds another interesting twist on some of the physiological changes induced by stress.

Of interest and relevant to the discussion of latent EBV reactivation is the observation by Valerie et al. (91), who demonstrated that radiation-induced cell suicide resulted in the increase of replication and reactivation of HIV. In other related studies, it has been demonstrated that certain viruses may be capable of modulating the ability of cells to respond to cytotoxic stress by the initiation of apoptosis (92). Since glucocorticoids have the ability to induce apoptosis in lymphocytes (93), a theoretical connection can be made among these observations and implications for stress-associated changes in virus replication and reactivation.

## VIII. CONCLUSION

In this chapter we have highlighted data from a variety of studies which includes medical students experiencing academic stress, separated and divorced men and women, psychiatric inpatients with major depression diagnosis, family caregivers of Alzheimer's disease victims, and on in vivo HSV-1 mouse model to explore the interactions among the CNS, endocrine, and immune systems and the physiological consequences induced by a variety of stressors on these interactions. In addition to demonstrating a down regulation of the cellular immune response in all these studies, many of them have also shown changes in the status of latent EBV and HSV concomitant with the immune changes.

It is not known how far "above or below" a mean level of immune competence (for normal individuals) the immune response must fluctuate in order to increase the risk for pathology. The question as to whether the interactions among the CNS, endocrine, and immune systems play a significant role in clinical outcomes of primary and latent herpesvirus infections is just now being addressed. The data are interesting and provocative, but, clearly, further studies need to be performed.

We would speculate that distress-related immunosuppression may have its most important health consequences in individuals whose immune function is already impaired prior to the stressor. Within this scenario, the stressor would then down-regulate an immune response which is already starting out at a lower baseline than a "normal" immune response. What comes to mind immediately are older individuals who are an at-risk group for infectious diseases, such as pneumonia and influenza virus infections. Another example would be AIDS patients, who are already severely immune suppressed because of HIV infection (94,95). In these individuals and other individual whose immune system is already down-regulated, emotional distress may affect morbidity and mortality of infectious diseases in general and herpesvirus infections in particular.

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