Stress-Related Activation of Epstein–Barr Virus

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Herpesviruses characteristically persist in a latent state in the body over the lifetime of an individual. Under certain conditions, any one of the herpesviruses can be reactivated. The mechanisms underlying the establishment of latent virus infection or viral reactivation are not well understood; however, it is known that the cellular immune response plays a very important role in the maintenance of latency and in virus reactivation. One of the factors thought to be associated with the reactivation of latent herpesviruses is psychological stress. Using an examination stress model with medical student subjects, we previously demonstrated the reactivation of latent Epstein-Barr virus (EBV), as measured by increases in antibody titers. In this follow-up study using the same group of medical students, we found evidence for incomplete reactivation of latent EBV, with only selective expression of the latent virus genome. © 1991 Academic Press, Inc.

Infection with one of the human herpesviruses often results in seroconversion in the absence of detectable clinical disease. In either the presence or the absence of clinical disease, however, each of the herpesviruses characteristically persists in a latent state in the body, presumably for the lifetime of that individual. Under certain conditions, any one of these herpesviruses can be reactivated to lytically replicate. Neither the mechanisms underlying the establishment of latent virus infection in the appropriate target cell nor the mechanisms underlying viral reactivation are well understood. It is known, however, that reactivation of the herpesviruses 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 suppression of latent virus and of the reestablishment of control over reactivated virus.

In normal individuals who have a well-functioning cellular immune response, reactivation of these viruses can occur sporadically, often in the absence of clinical disease. Reactivation may be detected by a noticeable increase in the specific antibody titer to the virus. This increase in antibody titer may occur simultaneously with the detection of infectious virus or in the absence of infectious virus. Furthermore, to complicate the issue, normal individuals who demonstrate an increase in antibody levels and/or the release of infectious virus may not have detectable virus-associated lesions or clinical symptoms (Sekizawa, Openshaw,

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Wohlenberg, & Notkins, 1980; Stevens, 1978). However, in patients who are severely immunosuppressed, such as renal transplant recipients, reactivation of one or more of the latent herpesviruses can result in severe morbidity and mortality (Armstrong, Evans, Rao, & Ho, 1976; Henle & Henle, 1985; Yao, Rickinson, Gaston, & Epstein, 1985; McVoy & Adler, 1989; Herrod, Dow, & Sullivan, 1983; Cheeseeman, Henle, Rubin, Tolkoff-Rubin, Cosimi, Cantell, Winkle, Herrin, Black, Russell, & Hirsch, 1980).

Continuous virus shedding that is not associated with disease is exemplified by persistent asymptomatic shedding of cytomegalovirus (CMV) in the urine or Epstein-Barr virus (EBV) in the saliva of normal individuals (Ho, 1982; Niederman, Miller, Pearson, Pagano, & Dowaliby, 1976). Shedding of Herpes simplex virus (HSV) in the absence of lesions can be observed periodically by the identification of infectious virus in the saliva or in vaginal secretions (Lindgren, Douglas, & Couch, 1968; Rattray, Corey, Reeves, Vontver, & Holmes, 1978). The importance of the cellular immune response vs. neutralizing antibody in the maintenance of virus latency is supported by evidence that a recurrent disease such as HSV can occur even in the presence of high levels of neutralizing antibody, as already mentioned (Sekizawa et al., 1980; Douglas & Couch, 1968; Zweerink & Stanton, 1981). In the case of HSV, impaired cellular immunity has been associated with both mild and severe clinical symptoms due to recurrence of HSV (Korsager, Spencer, Mordorst, & Andersen, 1975), as well as an increase in recurrent disease (Donnenberg, Chaikof, & Aurelian, 1980; Sheridan, Donnenberg, Aurelian, & Elpern, 1982).

It is generally accepted that the cellular immune response is down-regulated as a result of aging. For example, antibody titers to EBV early antigen (EA) and virus capsid antigen (VCA) IgG and IgA were higher in older individuals compared to a younger group of medical students (Glaser, Strain, Tarr, Holliday, Donnerberg, & Kiecolt-Glaser, 1985b). In a more recent study, a similar age-related increase in antibody titers to CMV was found (McVoy & Adler, 1989).

One of the factors thought to be associated with the reactivation of latent herpesviruses is psychological stress. Using an academic stress model involving medical students, we demonstrated the reactivation of EBV and HSV as measured by increases in antibody titers to both viruses (Glaser, Kiecolt-Glaser, Speicher, & Holliday, 1983a; Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987). In one of the studies with the medical students, there was a reduction in EBV-specific T-cell immunity and the modulation of a HSV-specific leukocyte migration inhibition factor (LIF); similar changes in LIF have been found in patients with recurrent HSV-2 infection (Sheridan et al., 1982). Psychological stress has also been associated with latent HSV reactivation and the appearance of lesions (Glaser et al., 1985b, 1987; Katcher, Brightman, Luborsky, & Ship, 1973; Kemeny, Cohen, & Zegens, 1989), as well as exacerbating EBV-associated mononucleosis (IM) in West Point cadets (Kasl, Evans, & Niederman, 1979). Since our laboratory and others have shown that stress can down-regulate several components of the cellular immune response, these data are consistent with what is known about the central nervous system modulation of the cellular immune response.
In this study we continued to explore the effect of psychological stress (examinations) on the reactivation of latent EBV in medical student subjects and demonstrated increased EBV IgG antibody titers in the absence of detectable latent EBV in exfoliated cells in the nasopharynx. We also found evidence of incomplete reactivation of latent EBV, with only a selective expression of the latent EBV genome.

METHODS

Subjects

Fifteen medical students at The Ohio State University College of Medicine volunteered to participate in studies concerning stress-associated modulation of the immune response. The 15 students had a mean age of 24.2 (SD = 4.3). There were seven males and eight females in the group. These subjects were a subset of a larger group of 40 medical students on whom immunological changes across the academic year have been previously described (Glaser et al., 1987). The subset of 15 students were chosen because their EBV VCA IgG antibody titers, as determined by immunofluorescence (IF), had shown the widest fluctuations in the prior study, and we were only able to assay a limited number of samples for this follow-up study that addressed more specific questions about EBV latency.

The curriculum during the first 2 years of medical school at The Ohio State University College of Medicine is such that examinations for all student subjects are given in several 2- to 3-day blocks across the academic year without major examinations at other times during the academic year. The immunological and self-report data were obtained at three “baseline” periods (lower stress sample points), approximately 1 month before the examination block and again during the examination blocks, as follows: September (baseline, A), October (examinations, B), January (baseline, C), February (examinations, D), April (baseline, E), and May (examinations, F). The January blood samples and self-report data were obtained immediately after students returned from the holidays, while the blood samples in April were drawn shortly after spring break.

Indirect Immunofluorescence Test for EBV VCA IgG

The indirect IF test to measure EBV VCA IgG was performed as previously described (Glaser et al., 1987). Briefly, acetone-fixed smears of P3HR-1 (HR-1) cells were adsorbed with twofold dilutions (in PBS) of subject plasma samples. After washing with PBS, the cells were adsorbed with goat anti-human IgG conjugated to fluorescein isothiocyanate, counter-stained with Evans blue, mounted in Protex, and examined using a Zeiss fluorescent microscope. The end point antibody titer for EBV VCA IgG was determined by the highest dilution of plasma showing detectable IF positive HR-1 cells. All assays were read blind coded.

Titration of Antibody to EBV Polypeptides by ELISA

Plasma was obtained from the blood samples (treated with EDTA) by low-speed centrifugation (1000g) for 10 min, and assayed for EBV antibody titers. The ELISA for measuring antibodies to individual EBV polypeptides was performed.
as previously described in detail (Pearson, Vroman, Chase, Sculley, Hummel, & Kieff, 1983; Kishishita, Luka, Vroman, Poduslo, & Pearson, 1984; Luka, Chase, & Pearson, 1984; Vroman, Luka, Rodriguez, & Pearson, 1985; Luka, Miller, Jornvall, & Pearson, 1986; Goldschmidt, Luka, & Pearson, 1987). Briefly, cell extracts were prepared from HR-1 cells expressing the different EBV antigens in an extraction buffer containing 0.5% NP-40 (Luka et al., 1984). Cell-free supernatants were then passed sequentially over affinity columns prepared with monoclonal antibodies (MAb) to four EBV polypeptides; the R3 MAb to the 52/50-kDa diffuse (EA-D) antigen, the K9 MAb to the 85-kDa restricted early antigen (EA-R) (two early proteins), the L2 MAb to the 125-kDa VCA protein, and the 2L10 MAb to the Gp 350/300 membrane antigen (MA) (two late proteins). The bound proteins were eluted from each column with 3 M MgCl2 in 20 mM Tris–HCl, pH 7.4, and dialyzed against 20 mM NH4HCO3 buffer overnight. The presence and purity of each EBV-specific polypeptide was then monitored by immunoblotting as previously described (Pearson et al., 1983; Goldschmidt et al., 1987). For preparing ELISA plates for measuring antibodies to the individual EBV polypeptides, 100 µl of a predetermined optimal concentration of the appropriate antigen was diluted in 0.5 M Na2CO3 buffer, pH 9.5, and incubated in wells of polystyrene microtiter plates (Linbro) overnight at 4°C (Luka et al., 1984). The plates were then washed twice with 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20 supplemented with 100 mg ovalbumin/liter (Sigma), dried for 20 min at room temperature, and stored dry at 4°C until used in the antibody detection experiments.

Twofold dilutions of the plasma samples were prepared in Tris, pH 7.4, buffer, containing 0.05% Tween 20 and 0.01% chicken ovalbumin (Sigma). A subset of 15 of the original subject sample of 40 was assayed for antibodies to the specific EBV polypeptides (Glaser et al., 1987); only 15 subjects could be studied due to the limited availability of the purified virus polypeptides. The plasma samples were incubated with each antigen for 1 h at room temperature. After incubation, the plates were washed with washing buffer and a 1:500 dilution of alkaline phosphatase-conjugated goat anti-human IgG (Sigma) was added. The plates were incubated for 45 min at room temperature and then washed. The color reaction was developed by adding 100 µl of phosphate buffer substrate (Sigma) dissolved in diethanolamine buffer, pH 10.4. The reaction was stopped by adding 50 µl of 3 M NaOH. Absorbance was read with a Titertech multiscan reader at OD 405 nm. Antibody titers were expressed as the reciprocal of the highest dilution which gave OD values 2 SD above the negative control plasma sample which was run on each plate. The plasma samples collected over the entire academic year for each student (six samples) were run at the same time and were blind coded.

Assay for the Presence of EBV DNA Positive Exfoliated Cells in Throat Washing Samples by DNA Dot Blot Hybridization

Subjects gargled with 100 ml sterile RPMI 1640 medium in order to obtain throat wash samples. The samples were centrifuged at 1000 rpm for 15 min in order to pellet the exfoliated cells. Total DNA extraction was performed using the cell pellets. EBV genome positive Raji and EBV genome negative BJAB cellular DNA
were used as controls; 5, 10, and 20 μg DNA from each cell line was applied to nitrocellulose paper. DNA blot paper was treated with blot buffer (1.5 M NaCl, 0.5 M NaOH) for 7 min and TN (0.5 M Tris–HCl, 1.5 M NaCl, pH 7.5) for 5 min three times, then baked at 80°C for 2 h. The DNA was hybridized with the BAMB1-W fragment labeled with P32-dATP and dTTP as previously described (Zhang, Yao, Zhu, & Glaser, 1991).

Anticomplementary Immunofluorescence Assay for the Detection of the Epstein–Barr Virus-Associated Nuclear Antigens (EBNA)

Antibody to EBNA has been shown to be a reflection of the specific T-cell response to EBV-transformed (latently infected) B lymphocytes (Masucci, Szigeti, Ernberg, Masucci, Klein, Chessels, Sieff, Lie, Glomstein, Businco, Henle, Henle, Pearson, Sakamoto, & Purtillo, 1981). In order to determine antibody titers to EBNA, the anti-complement IF test was used, using smears of Raji cells prepared on coverslips, fixed in acetone/methanol (50:50) for 10 min (Reedman & Klein, 1973). Twofold dilutions of the plasma samples were used to determine the anti-EBNA antibody titers blind coded, using a Zeiss UV microscope.

RESULTS

Self-Report Data

As reported in the previous study (Glaser et al., 1987), there were cyclical changes in academic stress, with the expected increases in distress found during examinations (Derogatis & Spencer, 1982). We also assessed a number of health-related behaviors that might have been associated with the observed changes in cellular immunity. Plasma albumin levels, a measure of nutrition, were within normal limits for all subjects, and there were no significant weight changes throughout the year. Alcohol use was minimal. Not surprisingly, students reported less sleep during examinations than they did at baseline; however, changes in sleep in this study and in other studies from our laboratory have not been reliably related to immunological data (Glaser et al., 1987). These variables were analyzed using repeated-measures ANOVAs.

Antibody Titers to the EBV VCA Complex (IgG) as Determined by IF

Antibody titers to the EBV VCA complex of proteins (IgG) were converted using a base 10 log to normalize the distribution, since they had been determined by twofold dilutions. A repeated-measures ANOVA showed a significant change in EBV IgG antibody titers across the six sample points, with higher titers found in plasma samples obtained during examinations. $F(5,10) = 8.33, p < .01$ (Fig. 1).

Attempt to Detect EBV in the Nasopharynx of the Medical Student Subjects

In order to determine if there was virus shedding associated with academic stress and changes in antibody titers to the EBV VCA complex of proteins, throat washing samples were prepared as described under Methods. Total DNA was extracted from the cells and examined by DNA dot blot hybridization for the presence of EBV DNA (as a measure of the release of virus). EBV DNA was
FIG. 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 as previously described (Glaser et al., 1987).

detected in one specimen of one subject at the time of examinations, and none was detected at baseline.

The Detection of Antibodies Directed to EBV-Specific Polypeptides

Since we were not able to detect EBV DNA in the throat washing samples in the same group of medical students showing a significant increase in EBV IgG antibody titers by IF, we tested the hypothesis that the reactivation of latent EBV, as demonstrated by the increase of antibody titers to EBV IgG by IF in the absence of detectable EBV DNA in exfoliated cells in the nasopharynx, was due to partial reactivation of the virus genome. The increase in antibody titers could be due to the expression of only certain viral genes, perhaps only genes involved in early function that do not require virus DNA synthesis. The viral proteins encoded by these genes would induce memory components of the cellular and humoral immune response to make antibody, more or less, to these gene products. The use of the indirect IF test would not permit us to discriminate between complete or incomplete reactivation.

In order to test this hypothesis, ELISA plates were prepared using two early and two late viral-encoded polypeptides that had been purified with EBV-specific MAbs as described under Methods. By using this procedure it was possible to determine whether there were any differences in the levels of one EBV-specific antibody to a specific viral-encoded polypeptide in each plasma sample.

We initially used 125 kDa VCA protein-coated plates. As shown in Fig. 2, there was not a significant change in antibody titers to this protein across the six blood samples using a repeated-measures ANOVA. A similar nonsignificant result was obtained using the GP 350/300 MA late protein and one of the early proteins (85
kDa) that is part of the restricted component of the EA complex, EA-R (Figs. 3 and 4).

When the 52/50-kDa EA-D polypeptide was used, changes in antibody levels were observed across the six blood samples (Table 1). The log₁₀ antibody titer to this polypeptide in those students who were seropositive for this protein increased from a baseline value of 1.11 ± 0.38 (September) to a value of 3.19 ± 0.49 at the time of examinations (October). The antibody titer stayed at this level until the next sampling obtained at the examination blood sample in February, when it leveled off at a level comparable to or below the titer observed at the start of the
study. We followed these initial analyses by examining the data nonparametrically. In examining the raw data for the 52/50-kDa EA-D protein, we found that the number of individuals who were positive for this protein changed dramatically across the sample points, with no subjects who were positive at one point. This necessitated the use of Cochran’s test, a nonparametric procedure for comparing frequencies involving repeated observations where the dependent variable can take on only two values (in this case, either positive or negative for the 52/50-kDa EA-D protein) (Cochran, 1950). Cochran’s test revealed significant differences in percentage of positive subjects across the bleeds, \( p < .0001 \) (Table 1). These data suggest either that the expression of the gene encoding the 52/50-kDa EA-D protein was "switched on/switched off" over time or that the expression of this gene, and the level of subsequent protein synthesis, was not at sufficient levels to induce the production of antibody detectable by ELISA. The data also suggest that the

![Image: 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 as described under Methods.](image)

**TABLE 1**

| Bleed       | N  | No. positive | % Positive | Antibody titers  
<table>
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<td></td>
<td></td>
<td></td>
<td>(log&lt;sub&gt;10&lt;/sub&gt;)</td>
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<tr>
<td>Baseline (Sept.)</td>
<td>15</td>
<td>6</td>
<td>40.0</td>
<td>1.11</td>
</tr>
<tr>
<td>Exam (Oct.)</td>
<td>15</td>
<td>13</td>
<td>86.7</td>
<td>3.19</td>
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<tr>
<td>Baseline (Jan.)</td>
<td>15</td>
<td>9</td>
<td>60.0</td>
<td>3.47</td>
</tr>
<tr>
<td>Exam (Feb.)</td>
<td>15</td>
<td>6</td>
<td>40.0</td>
<td>1.20</td>
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<tr>
<td>Baseline (April)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>.00</td>
</tr>
<tr>
<td>Exam (May)</td>
<td>15</td>
<td>2</td>
<td>13.3</td>
<td>.37</td>
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*Note. Cochran’s test; \( Q = 30.0; p < .001 \).*
gene encoding this polypeptide was "activated" between the first baseline and examination blood samples, then down-regulated over the rest of the study.

**Antibody Titers to EBNA**

We previously showed a down-regulation of the EBV-specific T-cell response in the same medical students from whom the plasma samples were obtained to perform the studies described in this report (Glaser et al., 1987). Since the level of antibody to EBNA is, at least in part, a reflection of the control of the outgrowth of B lymphocytes by cytotoxic T lymphocytes, these plasma samples were also examined for antibody to EBNA as part of this follow-up study. As shown in Fig. 5, no significant changes in antibody titers to EBNA were observed across the six bleeds. These data suggest that the down-regulation of EBV cytotoxic lymphocytes previously observed in these medical students was not sufficient to alter antibody titers to EBNA.

**DISCUSSION**

In this study we explored the effect of examination stress on the reactivation of latent EBV in medical student subjects and demonstrated increased EBV IgG antibody titers in the absence of detectable latent EBV DNA in exfoliated cells in the nasopharynx. We also found evidence of incomplete reactivation of latent EBV, with only selective expression of the latent EBV genome.

A previous study reported excellent correspondence between the detection of EBV DNA by DNA dot blot hybridization in exfoliated cells obtained from throat washing samples compared to the detection of infectious EBV through growth-transforming studies of B lymphocytes that used saliva as a source of infectious virus excreted in immune-suppressed patients (Diaz-Mitoma, Preiksaitis, Leung, & Tyrrel, 1987). Thus, dot blot hybridization is sensitive enough to detect EBV

![Graph Image](image-url)

**Fig. 5.** Antibody titers to EBNA across three baseline and three examination blood samples. Antibody titers were determined using the anticomplement IF assay as previously described (Reedman & Klein, 1973).
shed in the nasopharynx. The data obtained using DNA dot blot hybridization in this study did not demonstrate EBV shedding in the nasopharynx in the exfoliated cells at the time antibody titers to the EBV VCA complex of proteins (IgG) increased. In a recent study with another group of medical students, we assayed similar preparations across three baseline and three examination periods for the presence of EBV using the polymerase chain reaction (PCR) method. We found that 56–62% of the throat washing cell samples obtained at each time sample were positive for EBV DNA. Plasma samples obtained at the same time as the throat washings were assayed for EBV antibody levels as described in this paper in order to compare titers to virus shedding. While these data are not yet available, this is a very high percentage of virus shedders when compared to a group of 30 EBV seropositive normal adults in whom only 20% of their cell samples were EBV DNA positive by PCR (J. Jones, unpublished data). The preliminary data from this study suggest that the release of EBV in the nasopharynx of these medical students did not coincide with examination periods, but may be related to the continuous stress of medical school. Whether antibody levels to EBV changed over time in Jones's study remains to be determined. However, as a group of individuals experiencing academic stress throughout the year, they had almost three times the incidence of EBV DNA positive exfoliated nasopharyngeal cells than the control group.

We considered the possibility that the changes in the antibody titers to EBV VCA IgG associated with stress that were obtained using the indirect IF test (in the absence of detectable EBV in the nasopharynx) could represent changes in one, several, or all EBV proteins being reactivated as a result of psychological stress at the time of examinations. If there were only selected proteins expressed as a result of partial reactivation of the viral genome, one would not expect to detect infectious virus or even defective virus particles containing EBV DNA in throat washing samples.

In order to explore this interesting possibility, we tested the hypothesis that not all viral genes were reactivated after the initiating event(s) to induce reactivation. These studies were performed using an ELISA. Using EBV-specific MAbs to both early and late EBV proteins, it was possible to prepare plastic plates coated with two early proteins, the 52/50-kDa EA-D and the EA-R 85-kDa proteins, and two late virus proteins, the 125-kDa VCA and the GP 350/300 MA proteins. The expression of proteins in the EA complex does not depend on EBV DNA synthesis, while the expression of the late viral proteins, VCA and MA, are dependent on EBV DNA synthesis. Thus, it was possible to probe for changes in the level of antibodies specific to one of these four proteins associated with EBV replication.

The data demonstrate that incomplete reactivation did occur in these medical students over the academic year. Supporting this interpretation are the data that show that the plasma samples with higher anti-VCA IgG antibody titers determined by IF (at the time of examinations). When examined for similar fluctuations in antibody levels to the 125-kDa VCA and GP 350/300 MA proteins, showed no changes in antibody titers to these two proteins. No evidence for changes in antibody titers to the early 85-kDa EA-R protein was found. However, there was
evidence that the early EA-D 52/50-kDa protein was modulated with an increase in the antibody titer between the September baseline and October examination periods; titers leveled off and then dropped for the remainder of the study. When the plasma samples were examined for the presence of antibody to the EA-D 52/50-kDa protein, individuals were found who did not have detectable antibody (<1:2) in a given blood sample, but became antibody positive in another blood sample, and some individuals who were antibody positive became negative over the course of the academic year. These data suggest that either the expression of the gene encoding for this protein was down-regulated sufficiently to cease the production of this protein or that the down-regulation of the production of the protein was sufficient to reduce the amount of antigen available to stimulate antibody production. Since the antibody titers to these four polypeptides did not coincide with the pattern observed by IF, it is possible that other proteins may be modulated in a selective way, that can be detected by IF and which were reactivated at different time points. Until additional EBV-specific MAbs are tested, it will not be possible to test this hypothesis.

EBV reactivity has been associated with depression (Allen & Tilkian, 1986; Pitts, Allen, & Allen, 1989; Greenberg, 1989) and with a chronic mononucleosis syndrome [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 and during the course of CFS, which makes a neuroimmune interaction a possible "cofactor" (Pert, Ruff, Weber, & Herkenham, 1985; Stein, Keller, & Schleifer, 1985; Straus, 1988). Consistent with this hypothesis, several of the immunological findings described in patients with CFS in reports by Straus (1988) and Jones and Straus (1987) include elevated antibodies to EBV, a decrease in responsiveness of lymphocytes to mitogens, decreased NK cell activity, and decreased EBV-specific cytotoxic T-cell activity. All of these responses have also been associated with psychological stress (Glaser et al., 1985, 1987).

In attempting to explain the mechanism(s) whereby latent EBV or other herpesviruses could be reactivated by stress-associated immune modulation, it is necessary to keep in mind that there is ample evidence that glucocorticoids, such as hydrocortisone, can induce latent EBV and can enhance CMV replication in vitro (Joncas, Boucher, Boudreault, & Granger-Julien, 1973; Prachova & Roubal, 1981; Bauer, 1983; Tanaka, Ogura, Kamiya, Sata, Yochie, Ogura, & Hatano, 1984). It has also been shown that glucocorticoids can enhance HIV replication in vitro as well (Markham, Salahuddin, Veren, Orndorff, & Gallo, 1986). It is known that glucocorticoids are released during psychological stress after the activation of the hypothalamus/pituitary/adrenal axis which occurs under stress (Cotman, Brinton, Galaburda, McEwen, & Schneider, 1987). We did not obtain glucocorticoid levels in these students due to the inability to arrange to get multiple samples; we did not believe that a single test sample would provide an accurate measure.

There is good evidence from in vitro studies that EBV can be either partially or fully induced to replicate from cells latently infected with the virus, after treatment of the cells with a variety of drugs. For example, only the EA complex is expressed in Raji cells after treatment with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) or iododeoxyuridine (I UdR); under these conditions
EBV DNA synthesis does not occur nor are late viral proteins, including VCA, synthesized. On the other hand, it is possible to induce early and late viral proteins, as well as the synthesis of infectious virus, in HR-1 cells treated with TPA or Iut (Glaser & Nonoyama, 1974; Hampar, Derge, Martos, Tagamets, Chang, & Chakrabarty, 1973; zur Hausen, O’Neill, & Freese, 1978). It has also been demonstrated that 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, Burkitt’s lymphoma (BL), and nasopharyngeal carcinoma (NPC) (Glaser & Zhang, 1985). There are examples of EBV-associated diseases in which antibodies to EBV DNA polymerase and DNase can be observed, such as CFS and NPC; antibodies to these enzymes, however, are not detectable in patients with IM or BL (reviewed in Glaser & Zhang, 1985).

These data, along with the virus activation studies in vitro already discussed, suggest that EBV gene regulation in vivo is not an all or none situation and that different gene products can be expressed under different conditions; in vivo, this could result in antibody patterns to specific gene products described above that have clinical relevance. Studies are presently underway in our laboratory using new MAbs to EBV polypeptides that will be used to purify other EBV-specific proteins, e.g., EBV DNA polymerase (Tsai, Williams, & Glaser, 1990a,b), to be used to further elucidate the pleiotropic reactivation of latent EBV found with the down-regulation of cellular immunity associated with psychological stress.

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