Herpes Viruses, Cytokines, and Altered Hemostasis in Vital Exhaustion

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Objective: Infections with herpes viruses have been implicated in the pathogenesis of atherosclerosis. We tested the hypothesis that vital exhaustion (VE) is associated with multiple herpes virus infections, such as herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus, and with an increase in pathogen burden (i.e., the aggregated seropositivity to immunoglobulin G antibodies for herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus). In addition, we examined the association of VE and pathogen burden with measures of hemostasis and inflammation. Methods: Blood samples were drawn from 29 men with VE and 30 male control subjects, all healthy and nonsmokers, to assess serological evidence of infection and measures of hemostasis and inflammation. Results: VE is associated with a relatively high pathogen burden, altered hemostasis, and higher levels of cytokines, such as interleukin-6. Across all subjects, a relatively high pathogen burden was also associated with altered hemostasis but not with increased cytokine levels. The interaction of VE with pathogen burden revealed significant linear increases in measures of hemostasis and inflammation. Finally, immunoglobulin G antibody titer levels of individual herpesvirus infections were not associated with hemostatic measures or with cytokines. Conclusions: We conclude that stress-related alterations in hemostasis and inflammation are not necessarily linked to one particular herpesvirus infection but rather to an increase in aggregated seropositivity to herpesvirus infections. Key words: herpesvirus infections, hemostasis, cytokines, vital exhaustion, stress, coronary artery disease.

INTRODUCTION

Chronic psychological stress has been related to impaired cellular immunity (1, 2). This may result in a stress-related increase in susceptibility to viral or intracellular infections, as was convincingly demonstrated in healthy individuals who were given nasal drops containing one of five respiratory viruses (3). That study showed that stress increases the risk of infectious respiratory illness in a dose-response manner. Stress-related reactivation of Epstein-Barr virus (EBV) infection has also been studied: significantly higher antibody titers and EBV excretion in throat washings were demonstrated (4, 5). Furthermore, chronic stress modulates antibody titers to herpes simplex virus (HSV) infection (6–8) and the specific immunity to varicella-zoster virus (VZV) infection (9).

The group of herpesviruses include HSV, VZV, EBV, and cytomegalovirus (CMV). These viruses are characterized by lifelong latent infections that may reactivate in the case of impaired cellular immunity. Although complete reactivation may lead to a serious systemic disease in immunocompromised individuals, asymptomatic shedding or incomplete reactivation of these herpesvirus infections has also been documented, even in apparently immunocompetent individuals (5, 10–12). Although those studies indicate that reactivation does not necessarily result in a full-blown clinical presentation, other less apparent manifestations may occur. For instance, herpesvirus infections have been implicated in the pathogenesis of vascular disease, by infecting the arterial wall, by altering vascular cell lipid metabolism, by induction of cytokines and growth factors, and by procoagulant effects on the vascular endothelium (13). Notwithstanding this evidence, an increased risk of coronary artery disease (CAD) has not consistently been found for each of these individual viruses (14–16). Recent studies have, therefore, focused on CAD risk and the aggregate number of atherogenic viruses to which individuals have been exposed during their life (i.e., the pathogen burden or PB) (17–19). Those studies not only showed that an increased PB raises the risk of CAD but also suggested that stress-related susceptibility to viral or intracellular infections is involved in pathogen-induced CAD (17).
It is increasingly recognized that atherosclerosis is an inflammatory disease (20) and that not all of its clinical and epidemiological features are explained by classic risk factors (21). Other factors that influence the inflammatory response system (IRS), like infection (17) and psychological stress (22), also need to be considered. Psychological stress, for instance, may contribute directly to vascular disease by modulating cytokine responses (23, 24) or hemostasis (25–29) or indirectly by reactivating latent infections and subsequently inducing procoagulant activity (17). An increased risk of CAD has also repeatedly been documented in individuals suffering from vital exhaustion (VE) (30–33), a state of undue fatigue that individuals reach when their ability to cope with chronic stress begins to fail (34–36). The aim of the present study is to test the hypothesis that VE is associated with increased seroprevalence rates to HSV type 1 and 2, VZV, EBV, and CMV, and with an increased PB. In addition, we examined the association of VE and PB with measures of hemostasis and inflammation.

METHODS

Participants

To locate male volunteer participants, names and addresses of a random sample of 1600 men in the age range of 40 to 65 years were obtained from the population registries of four towns in the province of Limburg, The Netherlands. In response to a letter explaining the purpose of the study, 577 men (36.1%) completed the initial screening inventory, which included the Maastricht Questionnaire (MQ) to assess feelings of exhaustion (37) and a health survey to evaluate current disease status, smoking status, and medication (38). On the basis of this information, 223 subjects were excluded because of self-reported medical disorders (eg, cardiac disease, hypertension, pulmonary diseases, gastrointestinal complaints, clinical depression, diabetes mellitus, rheumatoid disorders, and Parkinson’s disease); 90 subjects were excluded because they were current smokers. From the remaining respondents, 54 potentially exhausted subjects (MQ score ≥18) and 33 control subjects (MQ score ≤7) entered phase two, in which the Maastricht Interview for Vital Exhaustion (MIVE) and the Structured Clinical Interview for DSM (SCID) were administered. The MIVE is a standardized interview of 23 questions designed to assess VE. Because the validity of the MIVE has been established in men only, no women were included in this study (39).

The SCID is a standardized interview to assess psychopathology according to DSM criteria (40). Only the part assessing major depression was used to lower the burden of the study for the participants. Subjects were classified as exhausted if 1) they endorsed more than 7 of the 23 MIVE items and 2) at least one of the elements of exhaustion (fatigue, irritability, or general malaise) had increased in intensity during the past 18 months. This procedure effectively excludes false-positives identified by the MQ in both healthy and CAD populations (39). On the basis of their responses during this second phase, 22 subjects were excluded because they failed to meet MIVE criteria of VE (N = 19) or because they met DSM criteria of major depression or other current mood disorders (N = 3). All control subjects met the selection criteria. Five selected subjects (two from the VE group and three from the control group) could not participate in the main study on the scheduled dates. Enrollment was discontinued when a group of 30 subjects with VE and 30 control subjects agreed to participate. One participant with VE, however, was excluded post hoc because of a subsequently detected hypercholesterolemia (cholesterol = 9.5 mMol/liter, normal = 4.1–6.4; high-density lipoprotein = 0.6 mMol/liter, normal = 0.6–1.9; LDL = 7.9, normal = 3.0–5.2). Thus, the final sample consisted of 29 participants with VE and 30 control participants, all nonsmokers and in good health. The study protocol was approved by the institutional review board, and all participants gave written informed consent.

During phase two, additional assessments were made of daily alcohol and coffee consumption and of weekly physical exercise (using a four-point scale ranging from “no regular physical exercise” to “daily physical exercise”). For validation purposes, assessments were also made of habitual sleep quality during the past 3 months (36), of major life events that might have occurred during the past 12 months (41), and of level of stress experienced during the last month (42).

Experimental Procedure

Participants slept 2 consecutive nights in sound-attenuated, single rooms. The first night was an adaptation night. After a day of usual activities, participants returned to the hospital at 5:30 PM. They refrained from consuming food, alcohol, and caffeinated beverages starting at 2:00 PM. Heart rate and blood pressure were measured after 15 minutes of bed rest. A light dinner (3614 kcal) was served at 7:00 PM. After an overnight fast, blood was drawn from an antecubital vein at 7:00 AM (with subjects in the supine position), before participants engaged in physical activity. Because blood sampling with vacuum tubes may induce sampling artifacts (43), an open system with a 1.2-mm needle was used, and minimal stasis was applied to the upper arm.

Laboratory Techniques

Serological evidence of infection. Blood samples were tested for immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to HSV type 1 and 2, VZV, EBV, and CMV. Blood was allowed to clot at room temperature and then centrifuged, and sera were stored at −70°C. Antibodies to HSV, VZV, and EBV were quantified by indirect immunofluorescence (Meridian Diagnostics Inc, Utah) per the manufacturer’s instructions. Seropositivity to these viruses is defined as an IgG value ≥10. For IgM antibodies, sera were pretreated with rheumafactor-sorbens (Dade Behring, Marburg, Germany) to remove this factor and interfering IgG. IgM cutoff values were also ≥10. Antibodies to CMV were quantified with the Microparticle Enzyme Immunoassay (Abbott Laboratories, Hoofddorp, Netherlands), using the Axsym automated analyzer. Seropositivity to CMV is defined as an IgG value ≥15 AU/ml. The IgM cutoff index value was ≥0.50.

Measures of IRS. Measures of IRS included interleukin-1 receptor-antagonist (IL-1ra), IL-6, IL-8, and IL-10. Blood was allowed to clot at room temperature, centrifuged, and stored at −70°C. Serum levels of all cytokines were quantified by means of enzyme-linked immunosorbent assay (ELISA) methods (Eurogenetics, Tessenderlo, Belgium), based on appropriate and validated sets of monoclonal antibodies (44, 45). The intraassay coefficient of variation (ICV) for all of these assays was <8%.

Measures of hemostasis. Measures of coagulation were prothrombin fragments 1 + 2 (F1 + 2), activated factor VII (FVIIa), factor VII and VIII coagulant activity (FVIIc, FVIIIc), von Willebrand factor antigen (vWFag), and fibrinogen. Fibrinolytic measures were tissue plasmin-
Blinded with respect to the psychological status of the participants.

VE and PB with hemostatic and IRS measures, three groups could be identified: a high stress, high PB group, an intermediate group, and a high stress, low PB group (see “Results”). These data were analyzed by one-way analysis of variance, and significant trends across groups were tested with polynomial contrasts. Furthermore, Bonferroni post hoc multiple comparisons were used to determine which specific means differed. Significance levels were based on two-tailed tests with a p value ≤.05.

**RESULTS**

Individuals with VE did not differ significantly from control subjects in age (VE: mean age = 51.1 years, SD = 4.5; control: mean age = 52.2 years, SD = 5.1; p = .4). Consistent with prior evidence, VE individuals reported higher levels of perceived stress, more major life events, and more habitual sleep problems than control subjects (all p values < .01) (34, 36, 47). They also drank more coffee per day (p < .01) but did not differ significantly from control subjects with respect to physical exercise, body mass index, heart rate, blood pressure, glucose, lipids, and routine blood chemistry values (all p values > .10).

**Vital Exhaustion and Serostatus**

None of the participants displayed evidence of a recent (active) infection, based on the absence of positive IgM antibodies to HSV, VZV, EBV, or CMV. Positive IgG antibodies were significantly more prevalent in VE individuals with respect to VZV (100% vs. 67%; p = .002) and CMV (79% vs. 37%; p = .001), whereas the groups did not differ significantly with respect to HSV (93% vs. 83%) and EBV (100% vs. 97%) (all p values > .10). Furthermore, PB was significantly higher in VE; four infections were documented in 72.4% of VE individuals and in 16.7% of control subjects, whereas two or three seropositive herpes infections were documented in 27.6% of VE individuals and in 83.3% of control subjects (Table 1).

**Vital Exhaustion and IgG Antibody Titer Levels**

In addition to serostatus, log-transformed titers were also compared between seropositive VE individuals and seropositive control subjects. In considering these seropositive participants, only the IgG titer levels of HSV were significantly higher in VE (Table 2).

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Vital Exhaustion, Hemostasis, and Inflammation

Significantly higher levels of fibrinogen and of all the fibrinolytic measures were found in individuals with VE (Table 3); a similar trend (data not shown) was observed for F1+2 (p = .06) and VII:c (p = .07). No significant group differences were found in VII:a, VIII:c, and vWF:ag (data not shown; all p values > .20). Furthermore, significantly higher levels of IL-1ra, IL-6, and IL-10 were found in individuals with VE, whereas no significant group differences were found in IL-8 (data not shown; p = .71).

Pathogen Burden, Hemostasis, and Inflammation

Significantly higher levels of all fibrinolytic measures and of F1+2 and VII:c (all p values < .04) were found in the high PB group (21 VE subjects, 5 control subjects) as compared with the low PB group (8 VE subjects, 25 control subjects). No significant group differences were found in fibrinogen, VII:a, VIII:c, and vWF:ag (all p values > .10). Furthermore, none of the cytokine levels differed significantly between the high and low PB groups (all p values > .10).

The Effect of PB and VE on Hemostasis and Inflammation

Three groups could be identified to investigate the association of VE and PB with measures of hemostasis and inflammation: a first group (N = 25) comprised control subjects with a low PB (low stress, low PB), a second group (N = 13) comprised either control subjects with a high PB or VE individuals with a low PB (intermediate), and a third group (N = 21) comprised VE individuals with a high PB (high stress, high PB). In comparing the low stress, low PB group to the intermediate and the high stress, high PB group, significant linear increases were found in fibrinogen, all fibrinolytic measures, and in IL-1ra, IL-6, and IL-10 (all p values < .01) (Fig. 1). Higher-order trends were not significant (all p values > .10).

Post hoc comparisons (with a p value of .05 ÷ 3 = \( \leq .017 \)) confirmed significantly higher levels in fibrinogen, in all fibrinolytic measures, and in IL-1ra and IL-10 in the high stress, high PB group compared with the low stress, low PB group (all p values < .017). A similar confirmation was not found for IL-6 (p = .05). The intermediate group was similar to the other two groups with respect to all of these measures (all p values > .10).

These same analyses were also performed using aggregated titer levels instead of using the aggregated number of seropositive tests: IgG antibody titer levels of HSV, VZV, EBV, and CMV were z-transformed and summed per subject. Using a median split, again three groups of individuals could be distinguished: individuals with low stress and a low aggregated titer level (N = 23), an intermediate group (N = 16), and individuals with high stress and a high aggregated titer level (N = 20). This approach revealed essentially the same results: significant linear increases were found in fibrinogen, in all fibrinolytic measures, and in IL-1ra, IL-6, and IL-10.
IgG Antibody Titer Levels, Hemostasis, and Inflammation

In considering seropositive participants, no significant associations were found between log-transformed titers of HSV (seropositive participants, N = 52), VZV (N = 49), EBV (N = 58), CMV (N = 35), and hemostatic or IRS measures (all p values > .10).

DISCUSSION

This study shows that seropositivity to the herpesviruses VZV and CMV was found significantly more often in exhausted individuals than in control subjects. A similar significant difference in the seroprevalence rates of HSV and EBV was not found between these groups. Furthermore, VE individuals had significantly more multiple herpesvirus infections, as indexed by their higher PB, than control subjects. These findings may be consistent with prior evidence indicating that chronic stress is associated with increased antibody titers to herpesvirus infections (4–8, 48), although it should be noted that only the IgG titer levels of HSV were significantly higher in seropositive VE individuals. The boosting of the antibody response in VE individuals, however, cannot adequately be explained by chronic stress itself since this causes a poor antibody response (49, 50), and additional studies are required to examine whether this boosting in exhausted individuals is due to reactivation of the virus itself.

The boosting of antibody titers may modulate production of cytokines such as IL-6 and IL-10 (51, 52), although none of these cytokine levels differed significantly between the high and low PB groups. This suggests that the increase in these cytokine levels is more likely the result of VE itself, consistent with prior evidence indicating a dysregulation of the inflammatory response system by chronic stress (23, 24, 53).

Because herpesvirus infections have been implicated in atherosclerosis (13, 17), measures of hemostasis were determined as well. The IgG antibody titer levels of individual herpesvirus infections were not associated with measures of hemostasis. A high PB, however, was associated with diminished fibrinolytic capacity (as reflected by the higher level of PAI-1 activity) and with increased activation of coagulation (as reflected by higher levels of F1+2 and VII:c). Moreover, VE was also associated with diminished fibrinolytic capacity and with an increased activation of coagulation (as reflected by the higher levels of fibrinogen, F1+2, and VII:c). This is consistent with prior evidence indicating that chronic stress alters hemostasis (25–29). This interaction of stress level and PB on significant linear increases in hemostatic measures therefore suggests that stress-related alterations in hemostasis are not necessarily linked to one particular herpesvirus infection but more likely to an increase in aggregated seropositivity to herpesvirus infections. These alterations in hemostasis may be caused by a direct effect of the herpesviruses on the vessel wall, but other mechanisms that do not require the actual local presence of the pathogen may mediate the procoagulant activity as well, like alterations in circulating levels of cytokines and acute-phase reactions. For instance, the significant linear increase between stress level/PB and IL-6 and the absence of such an association with von Willebrand factor support the notion that a systemic effect is more likely to be involved than a direct endothelial involvement.

This study was conducted in apparently healthy, nonsmoking individuals who suffered from undue fatigue, increased irritability, and general malaise. In cardiac populations, it was previously shown that VE cannot adequately be explained by (sub)clinical manifestations of CAD such as angina pectoris, extent of coronary atherosclerosis, or impaired left ventricular function (54). Our results indicate that VE is associated with multiple herpesvirus infections, increased procoagulant activity, and increased levels of cytokines. Cytokines, in particular, are relayed to the brain, where they evoke feelings of fatigue, general malaise, and feelings of lack of well-being (55). Although all of our findings may be directly related to chronic stress itself, we suggest a circular model in which chronic stress...
results in multiple herpesvirus infections, which amplifies the feelings of distress through the release of cytokines. The present cross-sectional study does not allow us to test this model, but our results do suggest that chronic stress is involved in the association between infection status and CAD risk. A prospective study, however, is needed to investigate the precise relation between chronic stress, infection status, and CAD risk.

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REFERENCES


We are saddened to announce the deaths of the following APS members within the past year:
Jerome H. Markovitz, MD, MPH ● Albert J. Silverman, MD ● Isao Tabeta, MD ● Herbert Weiner, MD DrMed ● Robin E. Wragg, MD

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