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Associations Between Stress, Trait Negative Affect, Acute Immune Reactivity, and Antibody Response to Hepatitis B Injection in Healthy Young Adults

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Eighty-four healthy graduate participants were administered the standard course of 3 hepatitis B vaccinations. Five months after the first dose (shortly after the second injection), each participant completed psychosocial measures, and a blood sample was drawn for determination of hepatitis B surface antibody titer. After completion of the vaccination series, participants performed an acute stress protocol, consisting of a 30-min adaptation period and a 5-min evaluative speech task. Blood was drawn at the end of the resting and task periods for assessment of cellular immune measures. Lower antibody response, as assessed after the second hepatitis B injection, was predicted independently by (a) high trait negative affect and (b) diminished T-cell proliferation in response to PHA. These data provide evidence that trait negative affect and the magnitude of stress-induced suppression of immune function may have clinical significance.

Key words: psychoneuroimmunology, cellular immune response, hepatitis B vaccination, acute laboratory stress, trait negative affect, naturalistic stress

A number of psychosocial parameters are thought to contribute to individual susceptibility to infectious disease. Of these, psychological stress is most often discussed and has been found associated with increased susceptibility to viral infections (Cohen, Tyrrell, & Smith, 1991, 1993; Stone et al., 1992) and greater severity of infection (Cohen et al., 1995). However, stress, per se, only accounts for a small proportion of observed variability in susceptibility. Hence, it is suggested that other characteristics may render individuals more or less vulnerable. One such attribute is the magnitude of stress-induced modulation of immune function. In this regard, it is well established that stress modulates aspects of immunity (Herbert & Cohen, 1993b). For example, acute laboratory stress alters both quantitative and functional components of cellular immunity, as indicated by alterations in peripheral T-suppressor/cytotoxic and natural killer (NK) cell populations and by a decreased ability of T lymphocytes to divide when incubated with nonspecific mitogens (e.g., Bachen et al., 1992; Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992). It has also been demonstrated that individuals differ appreciably in the magnitude of their immune responses to acute stress (e.g., Manuck, Cohen, Rabin, Muldoon, & Bachen, 1991) and that this interindividual variability is reproducible on retesting (Marsland, Manuck, Fazzari, Stewart, & Rabin, 1995; Mills, Haeri, & Dimsdale, 1995). Hence, it is conceivable that individual differences in immunologic reactivity to stress provide a marker of susceptibility to immune-related disease.

To date, one published study has explored the possibility that immune reactivity to stress predicts susceptibility to upper respiratory infection (Boyce et al., 1995). These investigators found that children, aged 3–5 years, showing the largest stress-induced increases in circulating B cells and pokeweed mitogen (PWM)-stimulated lymphocyte proliferation were at greatest risk for developing upper respiratory infections in response to an environmental stressor. However, it remains unclear whether these results generalize to adult populations and whether the type or magnitude of stress-related immune modulation is predictive of infectious susceptibility.

Another characteristic that could render individuals more or less vulnerable to infectious disease in the face of stressful events is the personality factor—trait negative affect, also referred to as neuroticism. This dispositional factor characterizes affective responses over long periods (months or years), is associated with increased

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vulnerability to stress (Costa & McCrae, 1985), and is thought to play a role in the onset and progression of physical disease (Watson & Pennebaker, 1989). Individuals high in trait negative affect are more prone to psychological symptoms of distress than persons scoring low on this dimension. This characteristic is also associated with compromised immune function, for example, lower proliferative responses to mitogens (Herbert & Cohen, 1993a). Therefore, it is possible that individuals high in trait negative affect are more susceptible to immune-mediated disease.

To begin to explore vulnerability factors that may influence the immune response to antigen, the following study investigates whether stress, immune reactivity, and trait negative affect predict antibody response to hepatitis B vaccination. Antibody response to this vaccination provides an in vivo measure of the competence of the immune system to respond when exposed to a novel antigen. Individuals vary substantially in their ability to mount and maintain an antibody response to hepatitis B vaccination, with approximately 10% of individuals failing to mount a protective response, 20–30% developing a low-level, transient response, and the remainder mounting larger, more robust responses (Pasko & Beam, 1990). A number of factors may contribute to such variability, including biological, behavioral, and psychosocial influences. Biological and behavioral factors associated with lower antibody titers include smoking, obesity, and being male and of older age (Craven, Awdeh, & Kunches, 1986; Horowitz, Ershler, McKinney, & Battiola, 1988; Klotz, Normand, & Silberman, 1986; Wood et al., 1993). Consistent with studies demonstrating that primary immune responses to novel antigens are subject to psychosocial influences (e.g., Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996; Snyder, Roghmann, & Sigal, 1993; Stone, Neale, Cox, & Napoli, 1994), attenuated antibody response to the hepatitis B vaccination series has also been associated with high levels of perceived stress at the time of initial antigen challenge (Jabaaij et al., 1993). Furthermore, Glaser and colleagues (1992) found that individuals who did not mount an antibody response to a primary challenge with hepatitis B antigen were more stress reactive (i.e., reported more stress in response to a subsequent examination period) than those who did seroconvert. The goal of the following study is to further investigate psychosocial factors that modify antibody response to hepatitis B vaccination, exploring whether variability in response is associated with life stress, trait negative affect, and/or individual differences in the magnitude of immune reactivity to stress. Finally, if stress or negative affect predicts vaccination response, then the possibility that immune reactivity moderates this relationship will also be explored. Here, findings that individuals vary consistently in the magnitude of their immune reactivity to stress make it conceivable that there is a meaningful distribution of differences in immunologic reactivity that may characterize individuals and form the physiological basis for differences in susceptibility to infection. Hence, high levels of stress or trait negative affect may have a greater impact on antibody response in more susceptible (immunoreactive) individuals.

Method

Participants

Participants were 84 healthy graduate students (51 men & 33 women) aged 21 to 33 years (M = 24). All participants were of normal weight and had no history or symptoms of systemic diseases known to affect the immune system. Participants received three 20-μg doses of recombinant hepatitis B vaccine (Heptavax B, SmithKline Beecham), administered intramuscularly into the deltoid muscle. The first two injections were given 6 weeks apart, followed by a booster injection at 6 months. All participants had received the first two immunizations prior to entry into the study. To make certain that participants had not been exposed to hepatitis B by infection or prior vaccination, we measured existing hepatitis B surface antigens and antibodies to hepatitis core antigen. No participant had serologic evidence of prior exposure. Therefore, the antibody to surface antigen measured in the current study was assumed to be subsequent to the two immunizations that they had received. Following completion of the vaccination series, participants attended a laboratory session lasting approximately 1 hr and scheduled at 8:00 or 9:30 a.m. At this time, measurements of cellular immune function and responses to the Profile of Mood States (POMS) questionnaire were obtained before and after participants performed a 5-min, videotaped speech task. Heart rate (HR) and blood pressure (BP) were also assessed at baseline and during task performance. All participants gave informed consent to participate in this investigation, which was approved by the Institutional Review Board of the University of Pittsburgh.

Procedures

In addition to vaccination appointments, participants visited the laboratory on two occasions for (a) a brief screening and (b) an acute stress protocol. Participants fasted for 12 hr before attending the screening session, which took place 5 months after the initial hepatitis B vaccination. At this visit, 10 ml of blood was drawn for the determination of antibody response to the hepatitis B surface antigen and serological markers of prior exposure to hepatitis B. Participants also received a battery of questionnaires to take home, complete, and return within a week.

Participants returned to the laboratory 2–4 months following completion of the hepatitis B vaccination series. The laboratory session was delayed 2 months following the vaccinations to assure that immune response to the antigen challenge would not influence acute immune reactivity. On this occasion, in order to control extraneous factors that can influence the immune system, participants were asked to abstain from alcohol for 24 hr and from food, caffeine, and exercise for 12 hr before testing. On arrival at the laboratory, an intravenous catheter was inserted into the antecubital fossa of the participant's right arm for the collection of blood samples, and an occluding cuff was placed on the left arm for automated measurement of HR and systolic (SBP) and diastolic BP (DBP; Critikon Dinamap 8100 vital signs monitor, Tampa, FL). Participants then rested quietly for a 30-min adaptation period. During the last 6 min of this period, baseline HR and BP (4 readings) were recorded, and 20 ml of blood was drawn for the determination of immune parameters, including reassessment of hepatitis B surface antibody levels. Participants then performed a simulated public speaking task, consisting of 2 min of preparation for a speech defending themselves against a hypothetical shoplifting charge, followed by 3 min of videotaped speech delivery. HR and BP were recorded every 90 s during speech preparation and performance (4 readings), and a second 20 ml of blood was collected immediately following task completion.

Hepatitis B Measures

Blood samples for the determination of hepatitis B surface antigen (HBsAG), and antibodies to hepatitis B surface and core antigens (anti-HBs and anti-HBc) were allowed to clot, centrifuged, and the serum was frozen at −80 °C until analysis. Antibody titers were determined by enzyme-linked immunoassay, using commercial kits (kindly donated by Abbott Laboratories).

Cellular Immune Measures

Functional immune measures included whole blood assessment of lymphocyte proliferative responses to phytohemagglutinin (PHA), concanavalin A (Con A), and PWM, establishing dose response curves at final concentrations of 2.5, 5.0, 10.0, and 20.0 μg/ml for PHA; 5.0, 10.0, 20.0, and 40.0 μg/ml for Con A; and 20.0, 100.0, 200.0, and 300.0 μg/ml for PWM. Response was defined as the difference in counts per minute between stimulated and unstimulated samples, determined separately for each concentration. Proliferation values were adjusted to take into account cell numbers. Peak mitogenic responses were found at doses of 10.0, 20.0, and 200.0 μg/ml for PHA, Con A, and PWM, respectively. Analyses were based on these optimal concentrations because prior comparisons involving use of a single, optimal concentration versus several mitogen concentrations (as in repeated-measures analysis) have been shown to yield similar results (Herbert, Coriell, & Cohen, 1994).

We assessed circulating numbers of T-cell subtypes, B-cells, and NK cells in whole blood using dual-color fluorescence analysis with a FACSCAN flow cytometer. Lymphocyte subsets were analyzed with monoclonal antibodies labeled with either fluorescein (FITC) or phycoerythrin (PE) to quantify CD3 + CD4 + (T-helper), CD3 + CD8 + (T-suppressor/cytotoxic), CD3 − CD19 + (B), and CD3 − CD16 + CD56 + (NK) cells. Isotope controls labeled with FITC or PE were used to assess nonspecific binding. Absolute numbers of cells were calculated from a complete blood count.

Psychosocial Measures

Participants were given a battery of questionnaires to complete at the time of the initial screening session. These measures provided an assessment of life event stress, perceived stress, trait negative affect, depression, and health behaviors. Life event stress was assessed by the Life Events List (LEL; Cohen et al., 1993), which provides a measure of the number of major stressful life events judged by the participant as having had a negative impact on his or her psychological state in the past year. The list contains events that might have occurred in the life of the participant (41 items) or those of others close to the participant (26 items). Participants recorded whether each of the events had happened to them and, if so, whether the event was a good or bad experience on a 6-point intensity scale. The 14-item Perceived Stress Scale (PSS; Cohen, Kamarck, & Marmelstein, 1983) was used to assess the degree to which the participant perceives that current demands exceed his or her ability to cope, evaluating the degree to which situations are appraised as stressful, with respect to dimensions of predictability, controllability, and overload. The LEL and PSS have been found to have acceptable levels of reliability and validity (Cohen et al., 1993; Cohen & Williamson, 1988).

An 88-item adjective rating scale was used to measure personality factors, including trait negative affect. The adjective list included entire subscales assessing trait negative affect taken from four well-validated instruments: anxiety and depression from the POMS Affect Scale (Usala & Hertzog, 1989), emotional stability from Goldberg's Big-5 Factor Scales (Goldberg, 1993), activated unpleasant affect and unpleasant affect from the Larsen and Diener Circumplex (Larsen & Diener, 1992), and positive loading for stress from the Mackay Circumplex (Mackay, Cox, Burrows, & Lazzerini, 1978). For each item, participants were required to rate accuracy of trait descriptions on a 5-point Likert scale ranging from 0 (*not at all accurate*) to 4 (*extremely accurate*). A principal components factor analysis with varimax rotation of the six trait negative affect scales was conducted. Anxiety (0.86), depression (0.83), emotional stability (−0.96), activated unpleasant affect (0.82), unpleasant affect (0.83), and positive loading for stress (0.90) loaded on a single factor. As a result, we created a negative trait affect scale by averaging the appropriate standardized scale scores and equally weighing each of the factors contributing to negative affect in the calculation of the overall scale score.

Finally, we used a modified version of the POMS to assess subjective responses to the speech task. For each item, participants were asked to indicate how they felt during baseline and task periods, on a 5-point intensity scale ranging from 0 = *not at all* to 4 = *extremely*. The modified POMS consisted of 25 adjectives, and responses yield subscale scores along 7 dimensions: fatigue, anger, anxiety, depression, vigor, well-being, and calm. Internal consistencies have been found to be high (Cronbach's αs = .84–.95).

Control Variables

A number of control variables were assessed that might provide alternative explanations for associations between stress or trait negative affect and response to hepatitis B vaccination. These included age, sex, body mass index, and depression, assessed by the Beck Depression Inventory (BDI; Beck, Ward, & Mendelson, 1961). Health practices including smoking (average number of cigarettes/day) and drinking alcohol (average number of alcoholic drinks/week).

Statistical Analysis

Antibody responses to hepatitis B surface antigen were measured at two timepoints, following both the second and third vaccinations of the series. Consistent with existing literature, 70% of participants had developed the maximal antibody response quantifiable by our methods (150 mIU/ml) following the third vaccination, and there was little variability across individuals. This ceiling effect prevented the examination of factors associated with individual differences in response. In contrast and as expected, an examination of the distribution of antibody responses following the second vaccination (5 months following the initial vaccination) revealed widespread interindividual variability (see Figure 1), with values ranging from no discernable antibody to the maximal response quantifiable in 25% of participants. For this reason and because past studies have shown that behavioral factors are associated with magnitude of antibody response around the time of antibody formation (e.g., Jabaaij et al., 1993; Snyder et al., 1993; Stone et al., 1994), we decided to focus on associations between psychosocial factors and antibody response following the second vaccination. Interestingly, the distribution of antibody responses at this timepoint was bimodal with most individuals falling at the extremes. For this reason, parametric analyses were not justified. Instead, we identified two groups of participants by dividing the distribution of antibody response at the median, permitting identification of individuals with High Ab or Low Ab responses (mIU/ml: Low Ab (n = 41): M = 16; High Ab (n = 40): M = 120). A series of logistic regression analyses was then performed examining whether psychological stress measures, trait negative affect, and/or immune reactivity predicted antibody response grouping. For these analyses, age, sex, and BMI were entered in the first step as standard control variables, followed by the independent variables (stress, trait negative affect, or immune reactivity) in the second step. To control for baseline covariation, the measures of immune reactivity were residualized values that resulted from regression of task measurements onto corresponding baselines to derive a covariance- (i.e., baseline) adjusted change score. Finally, we explored models of immune reactivity as a moderator of relationships between psychosocial variables and antibody response, again using logistic regression. For these analyses, the standard control variables were entered in the initial step, followed by both the psychosocial parameter of interest and immune reactivity in the second step. The terms for the interaction between the stress and immune measures were entered in a third step.

Results

Effectiveness of the Speech Task as an Experimental Stressor

To evaluate the effects of the speech task on cardiovascular, immune, and affect parameters, we compared mean baseline and

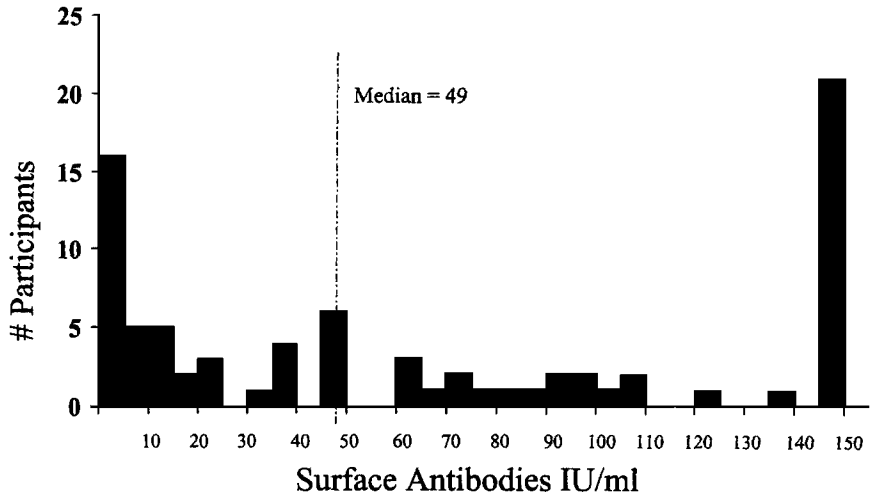

Figure 1. Frequency distribution of antibody response to hepatitis B vaccination at month 5.

task values within subjects using a series of *t* tests. A significant change from baseline to task measures was observed for all variables except B and T-helper lymphocytes (Table 1). Significant increases relative to baseline were found for HR, SBP, DBP, and total T, T-suppressor/cytotoxic, and NK cell numbers, whereas proliferative responses to PHA, Con A, and PWM decreased from baseline values. With respect to self-reported affect, analyses revealed increases relative to baseline for anger, $t(80) = -6.23, p < .00001$; anxiety, $t(80) = -11.28, p < .00001$; depression, $t(80) = -2.09, p < .04$; and vigor, $t(80) = -8.55, p < .00001$; whereas measures of fatigue, $t(80) = 12.02, p < .00001$; well-being, $t(80) = 7.70, p < .00001$; and calm, $t(80) = 15.77, p < .00001$, decreased from baseline values.

Comparison of Antibody Groups on Control Factors

We conducted preliminary analyses using *t* tests to compare the two antibody response groups on a number of control measures previously reported to influence magnitude of antibody response (Table 2). High and Low Ab participants did not differ in mean age, sex distribution, alcohol use, smoking status, or depression, as measured by the BDI. Consistent with previous findings, individuals in the Low Ab group had greater mean body mass index than their High Ab counterparts, $t(79) = 2.17, p < .03$. For this reason BMI was entered as a covariate in all subsequent analyses.

Stress, Negative Affect, and Antibody Response

Contrary to expectations, regression analyses revealed no main effect of state measures of stress, including stressful life events and perception of stress, on antibody response to the vaccination. With respect to trait negative affect, an inverse relationship with antibody response was predicted. Figure 2 presents trait negative affect among High and Low Ab participants. As apparent from the figure, individuals who mounted lower antibody responses to hepatitis B vaccination reported higher levels of trait negative

Table 1
Mean Baseline and Posttask Immune and Cardiovascular Measures

	Baseline		Task	
Measure	*M*	*SD*	*M*	*SD*
PHA (CPM × 1000)	1,262	619	1,084	500**
CON A (CPM × 1000)	756	324	678	286**
PWM (CPM × 1000)	848	407	712	344**
CD3 (cells/mm^3)	1,263	406	1,326	419*
CD4 (cells/mm^3)	760	312	768	313
CD8 (cells/mm^3)	462	156	503	173**
CD19 (cells/mm^3)	232	93	235	102
NK (cells/mm^3)	163	90	323	181**
HR (bpm)	63	10	77	14***
SBP (mmHg)	115	10	130	13***
DBP (mmHg)	64	8	77	9***

Note. PHA = phytohemagglutinin; CON A = concanavalin A; PWM = pokeweed mitogen; NK = natural killer; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure.
* $p < .05$. ** $p < .0001$. *** $p < .00001$.

Table 2
Characteristics of Low and High Ab Groups

	Low Ab		High Ab	
Characteristic	*M*	*SD*	*M*	*SD*
N	41		40	
Antibody Level				
Time 1	16	17	120	35***
Time 2	92	61	150	1***
Age	25	3	24	3
Sex				
Male	29		22	
Female	12		18	
No. alcoholic drinks/week	3.6	4.7	3.4	4.0
No. active smokers	2		4	
Exercise Index	−0.13	0.83	0.14	1.14
Body Mass Index	24.7	4.5	22.8	2.9*

Note. Low Ab = low antibody response; High Ab = high antibody response.
* $p < .05$. *** $p < .00001$.

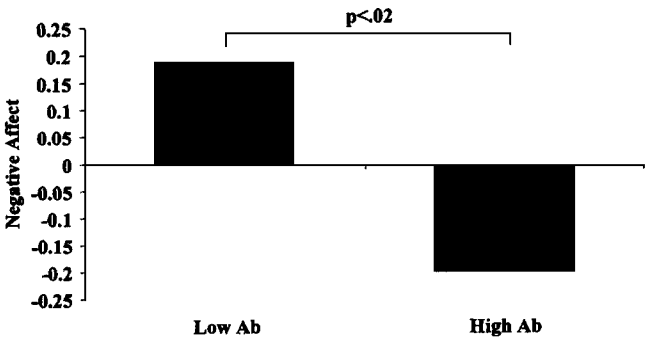

Figure 2. Trait negative affect (standardized score) among low antibody response (Low Ab) and high antibody response (High Ab) participants.

affect, with negative affect predicting antibody response group when the three control variables were entered into the equation ($b = -0.65$, $p < .02$).

In the above analyses, each of the measures was evaluated in separate models. However, the high correlations of trait negative affect and perceived stress ($r = .77$, $p < .00001$) and trait negative affect and life events ($r = .39$, $p < .04$) demonstrate that these scales are not independent. Hence, we performed a further analysis entering the two stress measures and trait negative affect into the equation in the second step. Negative affect was the only reliable predictor in this equation ($b = -.92$, $p < .04$), remaining a significant predictor of antibody response independent of the other stress measures. Finally, there was no relationship between depression, as measured by the BDI and antibody response.

Immune Reactivity and Antibody Response

With regard to functional measures of immunity, proliferative responses to PHA declined more between baseline and task measures in individuals who mounted lower antibody responses to hepatitis B vaccination ($b = .000001$, $p < .04$) than among their High Ab counterparts (see Figure 3). In contrast, proliferative responses to Con A or PWM were unrelated to antibody response ($ps > .40$). For cell subtype data, there was a tendency for stress-related changes in T-helper cells to predict antibody response classification, with helper cells decreasing among Low Ab participants when compared with the High Ab group ($b = .004$, $p < .09$). Otherwise, changes in circulating numbers of total T, T-suppressor/cytotoxic, B, and NK cells associated with the speech task were unrelated to antibody response ($b = .002$, $p < .19$; $b = .004$, $p < .15$; $b = -.0009$, $p < .85$; and $b = .002$, $p < .18$, respectively).

Immune Reactivity as a Moderator of Relationships Between Psychosocial Variables and Antibody Response

Finally, the possibility that immune reactivity moderated associations between trait negative affect and vaccination response was explored. Contrary to expectations, there was no significant effect of adding the interaction between negative affect and immune reactivity to any of the regression models. The results indicated that negative affect ($b = -0.16$, $p < .01$) and residualized change in proliferative response to PHA ($bs = .000001$, $ps < .03$) independently influence classification of individuals into the antibody response groups. No other significant effects were observed.

Discussion

The present study provides evidence for an association between two characteristics, trait negative affect and cellular immune reactivity, and magnitude of antibody response to hepatitis B vaccination. Consistent with studies demonstrating that primary immune responses to novel antigens are subject to psychosocial influences (Snyder et al., 1993; Stone et al., 1994), we found that participants who reported higher levels of trait negative affect had

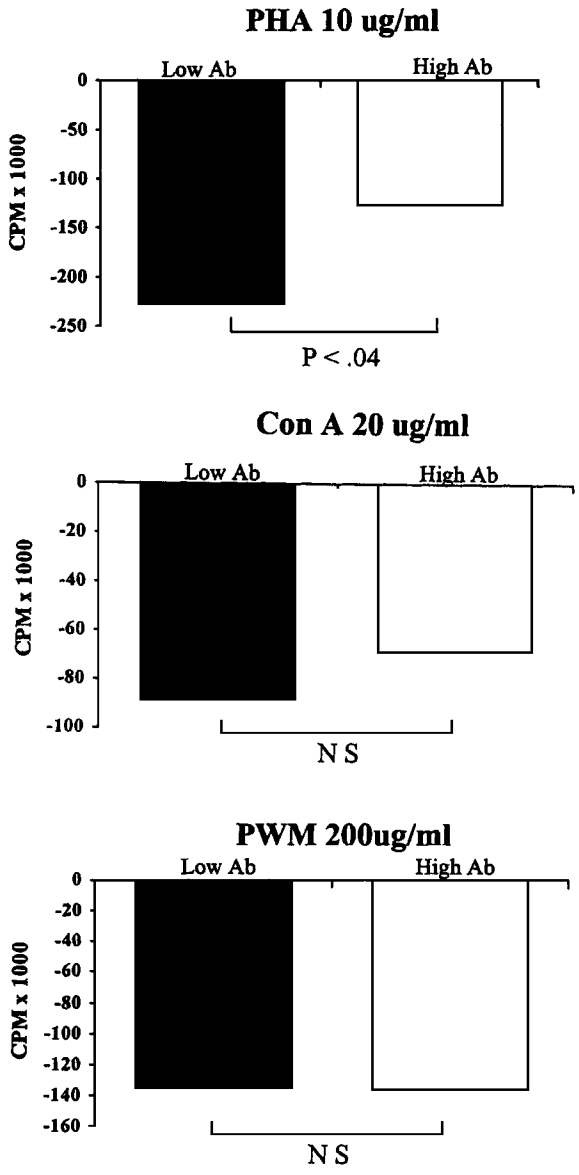

Figure 3. Baseline and age, sex, and weight-adjusted change in proliferative response to phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) among low antibody response (Low Ab) and high antibody response (High Ab) participants.

lower antibody responses to hepatitis B vaccination, as measured during the secondary antibody response. This finding supports an earlier report of delayed primary antibody response to hepatitis B vaccination among individuals who characteristically show high negative affect in response to examination stress (Glaser et al., 1992).

Consistent with existing literature, participants also showed a reduction of PHA-, Con A-, and PWM-stimulated T-cell proliferation and alterations in various circulating lymphocyte populations (increased peripheral T-suppressor/cytotoxic and NK cell numbers and reduced B cell population) following the speech stressor. A novel observation in this investigation is that the reduction of immune function, as measured by proliferative response to PHA, but not Con A or PWM, was greater in participants who mounted lower antibody responses to hepatitis B vaccination than among high antibody responders. Hence, this study provides initial evidence that individual differences in the magnitude of stress-induced reduction of immune function may be of clinical significance, being related to an in vivo immune response relevant for protection against infection.

Trait Negative Affect and Antibody Response

Although many theoretical and methodological issues remain, there is a large literature relating personality traits to health. Research on personality characteristics that make an individual more vulnerable to disease implicate negative emotional states (see Taylor, 1990, for a review). Indeed, there is a consensus that individuals with high levels of trait negative affect report more symptoms of disease than their low affect counterparts (Cohen et al., 1995; Costa & McCrae, 1985; Watson & Pennebaker, 1989). However, it remains unclear whether these associations reflect differences in underlying disease or biases in symptom reporting (Cohen et al., 1995). The present findings support a link between trait negative affect and an objective health measure, antibody response to vaccination, raising the possibility that in addition to reporting greater numbers of symptoms, individuals high in trait negative affect or neuroticism may mount less protective immune responses.

One possible moderator of associations between dispositional negative affect and antibody response to hepatitis B vaccinations is stress-related immune reactivity. In this case, stress-related immune reactivity would predict antibody response only in those with high trait negative affect. Although the current findings support wide interindividual variability in the magnitude of immune responses to laboratory stress, there was not significant interaction between trait negative affect and immune reactivity. Hence, the relationship between trait negative affect and antibody response observed in this study was not moderated by individual differences in immune reactivity to acute stress.

This study measured differences in antibody levels in the plateau phase of the secondary antibody response to hepatitis surface antigen vaccine. At this time in the antibody response memory B-lymphocytes are being maintained in germinal centers of lymphoid follicles, where their progeny are becoming antibody producing plasma cells. The differences in antibody titer probably reflect differences in the numbers of plasma cells produced from memory B cells. The number of memory B cells may, in turn, depend on the function of dendritic cells and helper-T lymphocytes that were involved in the initiation of antibody production. Thus, because of the complexity of the immune response, it is not possible to characterize the particular aspects of the immune system that were affected by the trait characteristics of the participants. There are, however, a couple of pathways that could underlie associations between negative affect and antibody response. First, there is evidence that the central nervous and immune systems are linked by neuroendocrine pathways. Alternatively, health behaviors could provide an indirect pathway by which trait negative affect could influence immunity. A number of health behaviors that modulate immune function, including sleep habits, exercise, diet, alcohol, and drug use (Kiecolt-Glaser & Glaser, 1988), accompany trait negative affect (Cohen & Williamson, 1988). Although the health practices measured in the current study were not related to antibody response, it is possible that other lifestyle variables, such as sleep habits or general activity levels, account for associations between negative affect and immunity.

Naturalistic Stress and Antibody Response

To date, the three studies exploring associations between state measures of perceived stress and antibody response to hepatitis B vaccination have yielded inconsistent findings. Studies measuring psychosocial factors around the time of initial antigen challenge find that stress and anxiety are associated with an attenuated (Jabaaij et al., 1993) or delayed (Glaser et al., 1992) primary antibody response to that antigen. In contrast, studies like the one reported here examining the impact of stress on the secondary antibody response find no significant (Jabaaij et al., 1993) or even opposite effects (Petry, Weems, & Livingstone, 1991). A similar pattern of findings is observed in studies examining antibody responses to in vivo challenge with other novel antigens (Snyder et al., 1993; Stone et al., 1994). Thus, there is some support for a negative impact of stress on the primary antibody response but not later in the immune response.

Cellular Immune Reactivity and Antibody Response

A question raised by associations between stress-induced immune reduction and antibody response is whether the decreased responsiveness of T lymphocytes to PHA is reflected in the reduced antibody response to the vaccine. PHA is a nonspecific mitogen that activates T lymphocytes to divide. As such, it provides an indirect measure of T lymphocyte function. One function of T cells is to activate B lymphocytes to produce antibodies specific to invading antigens. Hence, antibody production by B cells is dependent on the activation of specific T-cell populations (Roitt, Brostoff, & Male, 1989). Activation of the sympathetic nervous system under conditions of acute stress is associated with decreased T-cell activity, as measured by PHA responsiveness, which, in turn, is likely to render individuals less able to mount an antibody response. In support of the sympathetic regulation of immune reactivity, the current data reveal an association between stress-induced HR acceleration, an indirect measure of sympathoadrenal activation, and concomitant decreases in proliferative response to PHA ($r = -.26, p < .03$), Con A ($r = -.35, p < .003$), and PWM ($r = -.33, p < .004$). These findings raise a possible alternative explanation for the relationship between stress-induced decrease in proliferative response to PHA and antibody response

that heart rate reactivity may predict both of these effects. However, this does not appear to be the case as further regression analyses revealed no relationship between residualized changes in HR and antibody response.

A similar pattern was observed for relationships between antibody response to the vaccination and Con-A induced proliferation; however, these findings did not achieve significance. One explanation for discrepancies across measures is the possibility that PHA stimulates different T-cell populations than does Con A and that antibody production to hepatitis B surface-antigen is more highly dependent on the activation of PHA-stimulated T cells. Finally, in regard to the lack of association between antibody levels and proliferative responses to PWM, or quantitative measures of circulating lymphocyte subtypes, there are fewer reasons to expect that these measures would be associated with magnitude of antibody response. Although PWM stimulates activation of B cells, which produce antibody, activation of this cell subtype is the final step in the antibody response, and activation of T cells is a more proximate determinant of antibody response. Although it was not expected that quantitative measures of circulating lymphocyte subsets would provide a measure of the functional capabilities of the immune system, there was a tendency for individuals who mounted a lower response to the vaccine to show decreases in circulating numbers of T-helper cells following stress, as compared with an increase in this measure among their high antibody counterparts. Antibody production by B cells is dependent on the activation of T-helper cells, and decreases in numbers of this cell subtype may lead to lower antibody production.

Finally, it should be noted that the clinical significance of the observed differences in magnitude of antibody response is not yet known. We conducted this experiment using young, healthy participants and a vaccination protocol designed to produce maximal immunity to hepatitis B in greater than 90% of individuals. Interestingly, although the majority of participants mounted an antibody titer that is considered to be protective against hepatitis B infection by the end of the vaccination series, a significant difference between the antibody response groups remained, with Low Ab participants having a lower final antibody count than High Ab participants (M = 92 vs. 150, respectively). Although magnitude of final antibody response has been demonstrated to be a determinant of the duration of hepatitis B vaccine-induced immunity (Pasko & Beam, 1990), the measure used in the current study is not sensitive to antibody levels greater than 150 mIU/ml. As a consequence, it is not possible to capture the full range of antibody responses and evaluate whether predictors of antibody response following vaccination two are similarly predictive following the final vaccination. If it could be shown that negative affect and immune reactivity also predicted final antibody responses, then this association may explain why some individuals do not maintain protective immunity. Finally, in order to conclude that attributes such as trait negative affect and immune reactivity are vulnerability factors for susceptibility to disease, prospective studies are required that use measures of individual difference to predict disease outcome.

Although it is likely that trait negative affect and magnitude of immune reactivity to stress precede the immune response to vaccination in this study, causation cannot be attributed solely on the basis of correlational data. Indeed, it remains possible that trait negative affect or reduced immune reactivity following stress is a consequence of decreased immunocompetence or of more frequent illness. Alternatively, trait negative affect, immune reactivity, and ability to mount an antibody response may be related to a third factor, such as genetic predisposition. A further limitation of the current study is the timing of the assessment of immune reactivity, taking place 2 months after completion of the vaccination series. We chose this design to avoid any influence of immune response to the antigen challenge on acute immune reactivity. However, it rests on the assumption that magnitude of immune reactivity is a stable characteristic of individuals. Although there is preliminary evidence to support this assumption, further research exploring the stability and cross-situational consistency of immune reactivity is necessary before it can be concluded that immune reactivity is an enduring dispositional attribute. In sum, in order to specify causation, prospective studies are necessary with antibody response being predicted from trait factors and immune reactivity. The laboratory session was delayed 2 months following the vaccinations to assure that immune response to the antigen challenge would not influence acute immune reactivity.

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