Relationship of Stress, Distress, and the Immunologic Response to a Recombinant Hepatitis B Vaccine

L. Jeannine Petry, MD, Larry B. Weems II, and John N. Livingstone II
Augusta, Georgia

Background. Many studies have investigated the relationship between psychosocial factors and the susceptibility to infectious diseases. Fewer studies have investigated the relationship between stress and the immunologic response to vaccines. A follow-up study was designed to investigate the relationships between stress, distress, and the antibody response to a recombinant hepatitis B vaccine.

Methods. Eighty-one seronegative medical students received a standard immunization protocol of a recombinant hepatitis B vaccine. Six months after the first dose, corresponding to the induction phase of immunization, each subject completed both the Survey of Life Experiences and the Symptom Distress Check List to assess levels of stress and distress during that period. Three months after the third dose, corresponding to the booster phase of immunization, each subject completed the same questionnaires and was also tested for a quantitative hepatitis B surface antibody titer. Correlations were statistically analyzed using Pearson's correlation coefficient and stepwise multifactorial regression analysis.

Results. Higher levels of negatively perceived stress, irascibility, depression, and anxiety during the induction phase of immunization were significantly associated with higher peak antibody titers. Together these psychosocial factors accounted for 5.8% of the variance, and were as strong a determinant of peak antibody titer as was age.

Conclusions. In addition to the known vaccine-related and biological factors, psychosocial factors appear to affect the immune response to a recombinant hepatitis B vaccine. The positive direction of the correlation raises the question of whether the effect of psychosocial factors on antibody formation is different from their effect on antibody function.

Key words. Hepatitis B; viral hepatitis vaccines; antigen, immune response; stress. *J Fam Pract 1991; 32:481-486.*

Many studies have investigated the relationship between psychosocial and physiological factors in human subjects. Studies have linked altered psychosocial status with cardiovascular,[1,2] rheumatologic,[3,4] and infectious diseases.[5–8] Yet it is only with infectious disease that much work has been done to determine a specific relationship between severity of psychosocial stressors and specific pathophysiologic consequences that may lead to increased disease susceptibility. Clover et al[6] found that dysfunctional families had a higher incidence of influenza B infection during an epidemic in Oklahoma. Foulke and associates[7] indicated that low maternal scores of family functioning were associated with higher rates of infant otitis media and respiratory illness that required a physician visit. Boyce et al[8] reported an increase in the severity of childhood respiratory illnesses in association with increased stress and family routines. Kiecolt-Glaser and colleagues[9] found that separated and divorced men had both a higher degree of distress and more work days lost due to infectious diseases than did sociodemographically matched married men.

With such associations in mind, many investigators have searched for specific alterations of immunocompetence that may allow increased rates of infection among individuals under stress. Alterations in both cellular and humoral immunity have been noted in subjects experiencing high levels of stress. Decreased lymphocyte cytotoxicity,[10,11] decreased lymphocyte response to mitogens,[12] and changes in viral-induced lymphocyte proliferation[13] have been reported as measures of altered cellular immunity in individuals with high levels of stress or distress. With respect to humoral immunity, studies have shown decreased antibody titers[14] and decreased salivary IgA secretion rates[15] among stressed individuals.

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From the Department of Family Medicine, Medical College of Georgia, Augusta, Georgia. Requests for reprints should be addressed to L. Jeannine Petry, MD, Assistant Professor, Department of Family Medicine EG-206, Medical College of Georgia, Augusta, Georgia 30912-3500.

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Attempts to investigate a possible relationship between stress and the immunologic response to vaccines have yielded inconclusive results. Two studies of young adults inoculated with influenza viruses disclosed no relationship between life-change stress and antibody titers.[10,16] Roessler et al, as reported in Jemmott and Locke,[5] investigated the relationship between life-change stress and the antibody response to a trivalent influenza vaccine, and showed a decreased antibody response to one of the three viruses in subjects with high levels of life stress. Another study reported by Jemmott and Locke,[5] in which college students were given a swine influenza vaccine, revealed that a moderate level of recent life-change stress was associated with higher antibody titers.

The present study was designed to investigate the presence and direction of relationships between perceived stress, distress, and the antibody response to a recombinant hepatitis B vaccine.

Methods

Eighty-five generally healthy male and female medical students in the third month of their sophomore year agreed to participate in a hepatitis immunization program at the Student Health Center of the Medical College of Georgia. This study was reviewed and approved by the institutional Human Assurance Committee, and informed consent was obtained from each subject. Subjects' ages were determined at the time of enrollment in the study. All subjects were initially screened for the presence of hepatitis B surface antibody by a standard qualitative method using the Ausab-ELISA (hepatitis B surface antibody enzyme-linked immunosorbent assay) test (Abbott Laboratories, Diagnostics Division, Abbott Park, Ill). An intradermal Candida challenge, which provides a qualitative measure of the delayed hypersensitivity response, was administered to each subject as a marker for cell-mediated immunocompetence.

Immunologic response was determined by measuring subjects' quantitative hepatitis B surface antibody titers after receiving a standard vaccination schedule[17] of a recombinant hepatitis B vaccine (Recombivax HB, Merck Sharp & Dohme, West Point, Pa). Each subject received 10 μg of recombinant hepatitis B surface antigen vaccine by intramuscular injection in the deltoid region at the beginning of the study and at 1 month and 6 months after the first dose. All vaccine used in the study was from a single lot (lot #1589N, expiration date May 28, 1989), and all subjects received each vaccine dose within 2 weeks of one another. Initial antibody formation occurs during a 6-month induction phase, as a response to the initial and 1 month doses. The third vaccine dose, given 6 months after the first dose, serves to boost the initial antibody response. Serum samples were obtained to determine peak antibody response 3 months after the final vaccine dose was administered. Titers drawn at this time are representative of the maximum seroconversion rate of the vaccine and of the peak antibody titer attained.[17,18] Quantitative hepatitis B surface antibody titers were determined using the Ausab-ELISA test. Serial dilutions of subjects' sera were measured against a known standard, and quantitative titer levels were calculated.

Stress levels were determined by using the Survey of Life Experiences, a 57-item self-report measure listing events commonly experienced in the study population of college students. Subjects indicated stressful events they had personally experienced, and the perceived negative, neutral, or positive impacts of these events on their lives, using a seven-point bipolar scale. The data from these questionnaires were used to generate scores representing positively perceived and negatively perceived stress levels.[19]

Distress levels were determined by using the Symptom Distress Check List. Subjects indicated their perceived frequency of 51 symptoms on a four-point scale. These frequency scores were converted by a standard factor-analysis method to derive scores for five distress scales that assessed degrees of somatization, obsessive-compulsiveness, irascibility (a measure of interpersonal sensitivity and hostility), depression, and anxiety.[20–22] These symptom dimensions have previously been shown to be reliable and valid measures of current psychological symptom status.[20–22]

Both instruments were administered 6 months after the initial vaccine dose, to reflect events and symptoms that had occurred during the induction phase of immunization. Both instruments were again completed at the end of the booster phase of the vaccine series, when peak antibody response was determined, to reflect events and symptoms that had occurred during the booster phase of the study.

Quantitative hepatitis B surface antibody titers were compared with reference to subjects' sex and delayed hypersensitivity responses using a two-tailed *t* test. Pearson's correlation coefficient was used to compare quantitative hepatitis B surface antibody titers with reference to subjects' ages. Distress scales from both testing phases were analyzed for reliability within the study population. Levels of stress were compared with scores on the distress scales using Pearson's correlation coefficient. Peak antibody titers were compared with reference to the levels of stress and the scores on the distress scales during both testing phases using Pearson's correlation coefficient. Sta-

Table 1. Response Rates of Previously Seronegative Medical Students to a Recombinant Hepatitis B Vaccine

Response Group	Titer Range (IU/L)	Number of Subjects (N = 81)	Percentage
Nonresponders	0	0	0
Inadequate	1–9	1	1.2
Low	10–99	3	3.7
Adequate	100–999	20	24.7
High	>1000	57	70.4

tistically significant correlations were further analyzed using a stepwise multifactorial regression analysis.

Results

A total of 85 subjects were enrolled, all of whom were negative for hepatitis B surface antibody at the beginning of the study. Three subjects failed to complete the first questionnaires and were excluded from further analysis. The remaining 82 subjects, or 96.5%, completed all phases of the study. An integer value for the hepatitis B surface antibody titer could not be determined for one subject; therefore, this subject's data were excluded from all study analyses.

The subjects' ages ranged from 21 to 37 years, with a mean age of 24.8 years (±2.8 years). Of the subjects, 92.6% were younger than 30 years of age, and 70% were male. Positive delayed hypersensitivity to the intradermal Candida challenge test was seen in 79% of subjects.

After completion of the immunization series, the hepatitis B surface antibody titers showed an expectedly wide distribution, ranging from 9 to 8907 IU/L (mIU/mL) (Table 1). The mean hepatitis B surface antibody titer was 2220 ± 2005 IU/L (mIU/mL) with a median titer of 1576 IU/L (mIU/mL). The seroprotective response level of the vaccine has been determined to be 10 IU/L (mIU/mL) or greater.[23] The protective response rate of the vaccine was 98.8% for this study population, with one subject having an inadequate response.

During the induction phase, the mean positively perceived stress level was 2.2 (±2.5), and the mean negatively perceived stress level was 4.3 (±4.4). During the booster phase, the mean positively perceived stress level was 3.7 (±3.5), and the mean negatively perceived stress level was 6.5 (±5.6). During both the induction and booster phases, the mean negatively perceived stress levels were higher than the mean positively perceived stress levels.

Table 2. Correlates of Peak Antibody Titer with Self-reported Psychosocial Factors

Induction Phase	r	P
Negatively perceived stress	.19	.040
Positively perceived stress	.08	.240
Somatization	.02	.440
Obsessive-compulsiveness	.06	.300
Irascibility	.30	.003
Depression	.26	.009
Anxiety	.23	.020

The reliability coefficients for the distress scales calculated for both phases of the study showed that, during the induction phase, the obsessive-compulsiveness, irascibility, and depression scales were the most reliable (α = 0.83–0.89) for this population. The anxiety scale had a reliability coefficient of 0.72. During the booster phase, all five distress scales were very reliable (α = 0.84 to 0.91) for this population. Mean scores for each of the distress scales were derived for both the induction and booster phases of the study. The mean scores for the somatization, obsessive-compulsiveness, and irascibility scales were higher in the booster phase than in the induction phase. The mean scores for the depression and anxiety scales were higher in the induction phase than in the booster phase.

There were no statistically significant relationships between peak antibody titer and the subject's sex or delayed hypersensitivity response to an intradermal Candida challenge. Older subjects, however, had lower peak antibody titers ($r = -.22$, $P = .02$).

During both the induction and booster phases of immunization, subjects who had high levels of negatively perceived stress had high scores on all of the distress scales ($r = .19$, $P < .04$; to $r = .81$, $P < .001$).

Subjects with high levels of negatively perceived stress during the induction phase of immunization had higher peak antibody titers than those who had lower levels of stress ($r = .19$, $P = .044$). Higher peak antibody titers were also seen in those who had high induction phase scores for irascibility ($r = .30$, $P = .003$), depression ($r = .26$, $P = .009$), and anxiety ($r = .23$, $P = .02$) (Table 2). The booster phase levels of stress and distress did not show a statistically significant correlation with the peak antibody titer.

A stepwise multifactorial regression analysis of statistically significant correlates showed that a subject's age and induction phase levels of negatively perceived stress, irascibility, depression, and anxiety made independent

Table 3. Stepwise Regression Analysis of Psychosocial Factors and Peak Antibody Titer

Factor	Multiple r	r^2	Adjusted r^2
Age	.222	.050	.037
Irascibility	.372	.139	.116
Negatively perceived stress	.395	.156	.123
Anxiety	.398	.159	.114
Depression	.400	.160	.104

contributions to the peak antibody titer. Together, these factors accounted for 10.4% of the variance in peak antibody titers (Table 3). Further regression analysis of the induction phase levels of negatively perceived stress, irascibility, depression, and anxiety showed that these psychosocial factors alone accounted for 5.8% of the variance in peak antibody titers.

Discussion

A relationship was seen between perceived levels of stress, distress, and the immunologic response to a recombinant hepatitis B vaccine. Subjects who reported higher levels of negatively perceived stress, irascibility, depression, and anxiety during the induction phase of the immunization had higher peak antibody titers.

Many factors are known to affect the immune response to a recombinant hepatitis B vaccine. They can be categorized into vaccine-related, biological, and, on the basis of this study, psychosocial factors.

Vaccine-related factors that affect the immune response to a recombinant hepatitis B vaccine include dosage, potency, storage, schedule, and route and site of vaccine administration.[17,18,24–27] Within this study population, vaccine-related variables were controlled for all subjects and would not have contributed to the variability of the peak antibody titers.

Biological factors that have been shown to affect the immune response to a recombinant hepatitis B vaccine include the subject's age, weight, smoking status, immunocompetence, and genetics.[17,18,27,28] The relationship of age to titer seen in this study population is consistent with results obtained from other studies.[17,27–29] Older subjects developed lower peak antibody titers than younger subjects.

The possibility of confounding may exist in this study since subjects were not controlled for all biological factors, although age and the delayed hypersensitivity response to an intradermal Candida challenge were controlled.

Several psychosocial factors examined in this study were found to have a relationship with the immune response to a recombinant hepatitis B vaccine. The peak antibody response was related to negatively perceived stress, irascibility, depression, and anxiety during the induction phase. Although scores for negatively perceived stress, positively perceived stress, somatization, obsessive-compulsiveness, and irascibility were higher during the booster phase than during the induction phase, the only significant correlations with peak antibody titer were seen during the induction phase. This is consistent with other studies that have shown that psychosocial stressors that occur during the initial antibody response in the induction phase are related to peak antibody response.[5] This relationship has not been seen with psychosocial stressors occurring during the booster phase of immunizations.

Stress levels in this study were assessed by use of a retrospective self-report questionnaire. Although prospective recording of events may be more accurate, it appears that it is the perception of the level and type of stress generated by events that is of importance, and not the actual number or type of events experienced. The Survey of Life Experiences used in this study provides a better assessment of relative levels of stress experienced by groups when the recall period is 6 months or less and when the subjects are well educated.[30] Both of these conditions were met in this study of medical students.

The distress scores in this study were derived by use of a self-report questionnaire. Although distress scores were not measured when subjects entered the study, it may be that it is the actual level of current distress that is of importance rather than a change in levels of distress. The distress-symptom variables used in this study have been demonstrated to show factorial invariance, or constancy, across the variables of age, sex, and all but the lowest of social status groups.[21]

Higher induction phase levels of stress and distress showed a positive correlation with the peak antibody response to a recombinant hepatitis B vaccine. This finding is consistent with other research, which has shown that moderate levels of life-change stress are associated with higher antibody responses to some vaccines.[5] This study also demonstrates that moderate levels of distress are positively correlated with higher peak antibody titers to this vaccine.

The multifactorial regression model consisting of age and induction phase levels of negatively perceived stress, irascibility, depression, and anxiety accounted for 10.4% of the variance in peak antibody titer. In a further regression analysis that did not include the biological

factor of age, these psychosocial factors alone accounted for 5.8% of the variance in peak antibody titer. This finding suggests that induction phase psychosocial factors are at least as strong a determinant of peak antibody titer as age is.

The apparent paradox of high levels of psychosocial stressors relating to an increased susceptibility to infectious diseases, yet also to a higher peak antibody response to a vaccine, may be resolved by examining several factors. These include the function of stress, the antigen load presented to the stressed individual, and a differentiation between antibody formation and antibody function.

First, stress is not invariably a detrimental process. As has been seen in patients with cancer or chronic and debilitating diseases, a certain amount of stress may be used to promote healing rather than to cause illness, and may mobilize the immune system to enhance its protective abilities.

Second, the relationship of stress to the immune response may vary depending upon the amount of antigen presented to the immune system. The small antigen load used in a vaccine may present a different challenge to the immune system than an infectious agent introduced in an antigen load large enough to cause clinical disease.

Third, the effects of stress and distress on antibody formation may be different from their effects on antibody function. When applied to vaccines, these concepts are described as *vaccine immunogenicity*, or the ability of the vaccine to induce antibody formation, and *vaccine efficacy*, or the ability of that induced antibody to function in response to exposure to a specific infectious agent. When applied to the increased susceptibility to infectious diseases seen in highly stressed and distressed individuals, the predominant alterations seen have been in the function of both cellular and humoral immunity more than in the actual unchallenged levels of lymphocytes or antigen-specific antibodies.[10–15] Factors influencing vaccine immunogenicity, or antibody formation, may be different from those influencing vaccine efficacy, or antibody function.

Induction and persistence of the protective immunity produced by a recombinant hepatitis B vaccine have direct clinical significance. Peak antibody titer is the main determinant of the duration of hepatitis B vaccine-induced immunity.[23,27–29,31] Psychosocial stressors present during the induction phase of immunity affect the individual immune response, or peak antibody titer. By increasing the peak antibody titer, these psychosocial stressors may be postulated to result in an increased duration of immunity. Although it cannot be implied that this would impart additional protection if the individual is exposed to the hepatitis B virus, it may have an effect on decreasing the necessity and frequency of booster vaccinations to maintain detectable protective antibody levels.

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