Stress-Induced Modulation of the Immune Response to Recombinant Hepatitis B Vaccine

RONALD GLASER, PHD, JANICE K. KIECOLT-GLASER, PHD,
ROBERT H. BONNEAU, PHD, WILLIAM MALARKEY, MD
SUSAN KENNEDY, PHD, AND JOHN HUGHES PHD

Each of a series of three hepatitis B (Hep B) inoculations was given to 48 second-year medical students on the 3rd day of a 3-day examination series to study the effect of academic stress on the ability to generate an immune response to a primary antigen. Those students who seroconverted after the first injection (25%) were significantly less stressed and anxious than those who did not seroconvert at that time. In addition, students who reported greater social support demonstrated a stronger immune response to the vaccine at the time of the third inoculation, as measured by antibody titers to Hep B surface antigen (HBsAg) and the blastogenic response to a HBsAg peptide (SAg).

INTRODUCTION

Ongoing studies in our laboratory and others have addressed the impact of both severe and commonplace stressful events on immune modulation (1–5). Data from studies with medical students have shown that a psychological stressor, academic examinations, is associated with down-regulation of natural killer (NK) cell activity (6), lower synthesis of gamma interferon by peripheral blood leukocytes (PBLs) after stimulation with concanavalin A (Con A) (7), altered expression of the interleukin-2 receptor (8), and impaired cellular immune system control of the reactivation of certain latent herpesviruses (7, 9). These studies are reviewed elsewhere (10).

In addition to these stress-related immunological changes, data from these and other studies have also shown that personal relationships can impact on immune function. Lonelier medical students had poorer immune function than their less lonely contemporaries (6, 9). Marital disruption, either through bereavement or divorce, has also been associated with decrements in immune function (2, 5, 11). Family caregivers of Alzheimer's disease victims who reported lower levels of social support at intake into a longitudinal study showed the greatest and most uniform negative immunological changes when they were followed up a year later (12). These data are consistent with evidence from large, well-controlled epidemiological studies that have demonstrated that ". . . .social relationships, or the relative lack thereof, constitute a major risk factor for health—rivalling the

From the Department of Medical Microbiology and Immunology (R.G., R.H.B., W.M., S.K., J.H.), Comprehensive Cancer Center (R.G.), Department of Psychiatry (J.K.K.-G.), Department of Internal Medicine (R.G., W.M.), The Ohio State University College of Medicine, Columbus, Ohio.

Address reprint requests to: Ronald Glaser, Department of Medical Microbiology and Immunology, The Ohio State University College of Medicine, 333 West 10th Avenue, Columbus, OH 43210.

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effects of well-established health risk factors such as cigarette smoking, blood pressure, blood lipids, obesity, and physical activity" (13).

Human hepatitis B (Hep B) infection is a major public health problem throughout the world. While previously associated with blood transfusions, it is now also commonly associated with the use of street drugs, as well as with close contact with secretions from an infected carrier. Antibody to the Hep B surface antigen (HBsAg) is known to be protective against Hep B infections. Therefore, since there is no effective treatment for Hep B infection, the development of the Hep B recombinant vaccine has been important in controlling this disease.

In this study, we explored the effects of psychological stress and social support on the ability of the immune system to respond to a primary antigen, a Hep B vaccine. The Hep B recombinant vaccine is a highly effective, safe vaccine (14). In clinical trials, at least 91% of normal, healthy adults who completed the three-dose vaccine regimen seroconverted and possessed antibody to the HBsAg, with younger adults responding more rapidly and with higher antibody titers than older adults (14). Based on the previous observations already discussed, we explored the possibility that stress and social support could modulate the medical students' ability to respond to this vaccine.

SUBJECTS AND METHODS

The subjects were 48 second-year medical students (25 men and 23 women) who ranged in age from 22 to 29 (mean = 23.31, SEM = 0.20). The subjects received free vaccinations as an inducement for participation. At the time of this study, these second-year students had already been followed for a year and a half as part of another longitudinal study on stress and immune function. Thus, we had considerable psychological data on these students from both the prior year, as well as the year in which this study was conducted.

Our vaccination regimen was based on the protocol from Merck, Sharp and Dohme clinical trials (14), with the first two of the 10-μg vaccine injections given a month apart, followed by a third or booster injection at 6 months. The 10-μg dose was chosen because it induced seroconversion in 97% or more of adults between the ages of 20 and 29 who completed the three-dose regimen (14).

Each injection was administered on the 3rd day of a 3-day examination series to maximize any effects of stress on the immunological response to the vaccine. The first injection was given in mid-November, the second in mid-December, and the third in mid-May. Students were injected at all time points with the same vaccine lot. Blood samples for analyses were drawn before each injection, and all students were assessed on the same day.

Antibody titers to HBsAG were determined by enzyme-linked immunoassay (EIA). Commercial AUSAB EIA kits were obtained from Abbott Laboratories, North Chicago, IL. Briefly, polystyrene beads coated with human HBsAg (subtypes ad and ay) were incubated at room temperature for 18 hours with serial two-fold dilutions of each test plasma sample. Positive and negative controls were also included with each test. After washing, HBsAg conjugated to biotin and rabbit anti-biotin antibody conjugated with horseradish peroxidase were incubated with the beads. After washing the beads and incubation with substrate solution (O-phenylene diamine 2 HCl and 0.02% hydrogen peroxide), absorbency (492 nm) of the controls and test plasma samples were determined.

Each set of a subject's samples were titrated for HBsAg antibody within a single assay on the same day. This permitted internal standardization of all antibody titers for each subject. Lot to lot variation of reagents was not found to be a factor in determining titers. Antisera with known titers were used to determine the International Units (IU)/ml of antibody in each sample.

Peripheral blood leukocytes obtained from the medical students immediately prior to each vaccine inoculation were cultured in vitro with varying concentrations of yeast-derived HBsAg pre-S2 peptide (SAg) in order to measure the cell-mediated immune response to the vaccine. All blastogenic assays were conducted with fresh blood samples. In each of three wells of a 96-well U-bottom plate (Nunclon), 1×10^5 PBLs were cultured in 100 μl of RPMI 1640 medium

supplemented with 10% heat-inactivated fetal bovine serum, 25 μg/ml streptomycin sulfate, and 25 units/ml penicillin, 2 mM glutamine, 0.225% (w/v) sodium bicarbonate, 20 mM HEPES buffer, and 2×10^{-5} M β-mercaptoethanol. Each well also contained SAg at concentrations of either 5 μg or 1 μg per ml. The cells were incubated at 37°C in 5% CO_2 for 120 hours at which time 1 μCi of tritiated thymidine (specific activity 6.7 Ci/mM; ICN, Irvine, California) was added in a volume of 50 μl. The cultures were incubated for an additional 16 hours and the cells were then collected on glass fiber filter disks using an automatic cell harvester (Skatron, Sterling, VA). Cellular incorporation of tritium was determined by liquid scintillation analysis. The same lot of antigen was used in all the assays.

Several psychological measures provided data on stress, anxiety, and social support. The Profile of Mood States (POMS) (15) is one of the best self-report measures for identifying and assessing transient, fluctuating mood states. The measure is widely used, has excellent normative data, and psychometrically is very strong in terms of both reliability and validity (15). We were particularly interested in the tension-anxiety scale, the most responsive scale in our medical student population to the short-term increases in distress associated with examinations. The POMS was administered immediately prior to each vaccine inoculation.

The Perceived Stress Scale (PSS) (16) is a 14-item scale that assesses global perceptions of stress and measures the degree to which individuals appraise situations in their life as unpredictable, uncontrollable, and overloading. Normative data are available from a national probability sample (17). The PSS was administered at the second and third inoculation points.

The Interpersonal Support Evaluation List (ISEL) is a 40-item scale measuring perceived availability of four kinds of social support: appraisal, belonging, tangible, and self-esteem. Higher scores indicate greater reported support (15). The ISEL was administered in April, a month before the third and final vaccination. The test-retest reliability for a 4-week interval for the student version of the ISEL is 0.87. Although the ISEL was administered a month before the booster inoculation because of timing issues in the larger longitudinal study, we have confidence that the scores are stable (16).

RESULTS

All students were tested for antibody to HBsAg at the start of the study. None of the 48 students was antibody-positive on the day the first vaccine injection was given.

One quarter of the student sample, 12 of the 48 students, seroconverted for HBsAg antibody between the first and the second injections (early seroconverters). We compared levels of anxiety in the antibody-positive students with the antibody-negative students, using a repeated measures analysis of variance (ANOVA). The analysis included two between-subjects variables, presence or absence of antibody and sex of subject, and one within-subjects variable, change in anxiety over the three inoculation points. As shown in Figure 1, subjects who seroconverted after the first vaccine injection were significantly less anxious than students who seroconverted later, $F(1,44) = 6.28$, $p < 0.02$. In addition, students' anxiety levels varied across the three points in time,

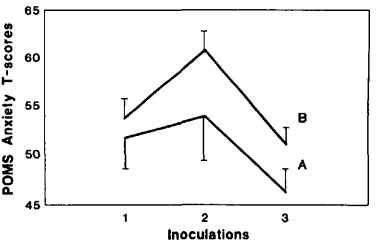

Fig. 1. Mean (±SEM) POMS anxiety t scores for medical students who had measurable antibody to Hep B at the time of the second inoculation (Group A, $n = 12$), compared with those students who had not yet seroconverted, and therefore were antibody negative (Group B, $n = 36$).

$F(2,43) = 6.33$, $p < 0.01$, with students reporting their highest levels of anxiety at the second inoculation (the second examination block of the series). There were no significant interactions among seroconversion group, sex, and change over time.

There were similar significant differences between the antibody positive and negative groups on the PSS, $F(1,43) = 4.22$, $p < 0.05$, with the early seroconverters having a mean PSS score of 23.58 (SEM = 2.41) at the second inoculation and 20.92 (SEM = 2.65) at the time of the third inoculation, compared with means of 28.17 (SEM = 1.46) and 22.77 (SEM = 1.25) in the later seroconvertors. The mean antibody titer of the 12 medical students who seroconverted after the first inoculation was 71.83 (SEM = 61.79). Since only 12 students had measurable antibody by the second inoculation, examining correlational relationships between the immune data and PSS or anxiety data was not meaningful. The early and later seroconverters did not differ on social support, $F < 1$.

The levels of stress and anxiety at the time of the second inoculation were noteworthy. Both the POMS anxiety t scores and the PSS scores at the time of the second inoculation for the entire sample were each approximately a standard deviation above general population norms (16, 17). Other data collected from the students as part of the larger study also suggest that this was a particularly stressful examination point. During the academic year, we had followed the student across periods when they had no examination. During three "baseline" periods, the mean for all students each time was between 42 and 43 on the POMS anxiety scale, or almost one standard deviation below the population mean. In addition, when we compared values for early and later seroconverters across the earlier academic year before this vaccine study, there were no differences on anxiety, perceived stress, social support, or the occurrence of recent stressful life events. Thus, we believe the differences are largely attributable to anxiety related to these particular examinations, although there were certainly other sources of tension or stress in students' lives as well.

By the third (booster) inoculation, 6 months after the first vaccination, all but one of the students had seroconverted, and the average antibody titer for all 48 students was 207.67 IU/ml (SEM = 31.30). The average antibody titer at the time of the third inoculation was 262.58 IU/ml (SEM = 54.72) for early seroconverters, while the average for the later seroconverters was 189.36 IU/ml (SEM = 37.45), but this difference was not statistically significant, $F(1,47) = 1.03$. Data were available from only 35 students for the blastogenic response of T lymphocytes to the SAg. Not surprisingly, the mean blastogenic response (counts per minute (cpm)) for the early seroconverters was higher than for the later seroconverters at both the second and third inoculation points. At the second inoculation the mean for early seroconverters was 9228.09 (SEM = 2517.32), while the mean for later seroconverters was 3794.06 (SEM = 2033.86). At the third inoculation, the mean blastogenic response of T lymphocytes obtained from the early seroconverters had means of 58,291 cpm (SEM = 19,344) for the 1 μg/ml concentration and 25,034 (SEM = 11,068) for the 5 μg/ml concentration, compared with 25,671 (SEM = 7,869) and 12,920 (SEM = 5,220) for the later seroconverters, but these differences did not reach significance, $F(1,33) = 2.78$, $p < 0.10$.

TABLE 1. Contributions of Anxiety and Social Support to the Immune Response to SAg

	Beta	Multiple r	R^2	t
Dependent variable: Antibody to HBsAg and blastogenic response to SAg (z-score), third injection				
Independent variables				
Step 1: Antibody titer, second inoculation	−0.02	0.07	0.00	0.92
Step 2: Blastogenic response, second inoculation	−0.35	0.26	0.07	−2.02
Step 3: POMS anxiety socres, third inoculation	−0.13	0.27	0.07	−0.71
Step 4: Social support	0.40	0.44	0.20	2.21*

*$p < 0.05$, $n = 35$.

We were interested in how stress and social support might have been related to the students' immune response to the vaccine in the interval between the second and third inoculations. To answer this question, we first computed the Pearson correlation between the HBsAg antibody titers and the peak blastogenic response of the PBLs to SAg at the booster (third) inoculation and found it was significant, $r = 0.53$, $p < 0.0001$. Moreover, a factor analysis with both variables produced a single factor with an eigenvalue of 1.53 that accounted for 76.3% of the variance. As discussed by Bray and Maxwell (18), highly correlated measures from the same domain can be combined to provide a single measure that better represents the immune response to the vaccine.

The immune response to an antigen involves a complex interaction of several populations of lymphoid cells, including the specific T-cell response and the production of antibody (to the antigen) by B cells, in this case, the Hep B vaccine. Since the antibody and the T-cell response to the vaccine are biologically related, we converted each student's data on these two variables to z-scores (since the measurement units are different for the two assays), and added the two z-scores together to provide a single summary measure of each student's immune response to the vaccine, with higher scores indicating a stronger immune response. The z-scores were used as the dependent variable in a hierarchical regression analysis (Table 1). We first entered the antibody titer and blastogenic response data obtained at the time of the second vaccination on the first step of the equation, thereby controlling for prior immune status. We then entered the students' anxiety scores on the second step of the equation, and social support was entered on the third step. As shown in Table 1, social support scores produced a significant increment in the variance after controlling for prior immune status and for anxiety, accounting for fully 13% of the variance in the immune response to the vaccine at the third inoculation. Anxiety did not produce a significant increment in the variance in this analysis; however, there were persistent differences in anxiety between early and late seroconverters, as shown in Figure 1. Although the greatest differences occurred at the second inoculation point, the group still had not converged by the time of the booster inoculation. Moreover, students'

anxiety scores for the second and third inoculations were strongly correlated, $r = 0.47$, $p < 0.001$, and lower anxiety had already been associated with earlier seroconversion. By controlling for the students' prior immune response to the vaccine, we were also effectively lowering the contribution for anxiety in this regression analysis.

In order to assess other health behaviors that might have had an impact on immunity, we collected information on recent sleep, weight change, alcohol use, and caffeine intake across the months of the study. There were not significant correlations between these data and the immunological data, consistent with prior studies from our laboratory (6, 9). Since the immune response can be altered by poor nutrition, we measured plasma albumin levels as a nutritional marker (19). Similar to the other health behaviors, nutrition was not significantly correlated with the Hep B antibody or blastogenic response; all students were within normal ranges in our study (data not shown).

DISCUSSION

In this study, we found that both stress and social support were related to medical students' ability to generate an antibody response to the HBsAg recombinant vaccine. Students who were more anxious and more stressed showed a delay in seroconversion. Following seroconversion, those who reported less social support had a poorer immune response to the HBsAg, as determined by both the combined measure for antibody titers and the T-cell response to Hep B SAg.

These data represent an important extension of past research on stress and immunity. The data show that stress and social support can modulate biologically-important responses in normal, healthy adults. If the timing of seroconversion to the HBsAg is affected by this relatively mild stressor in a student sample that has had ample prior experience with examinations, then the effects of more intense and more novel stressors on the response to the HBsAg vaccine and to other vaccines might have an even more profound effect.

Consistent with these data, a recent study utilizing a murine model of influenza virus infection showed that restraint stress altered the time of seroconversion and affected the kinetics of the synthesis of IgG and IgA to the virus (20). In another study, reduced serum antibodies were associated with social defeat in rats (21). Thus, convergent data from both human and animal studies suggest that stress may delay development of an immune response to a pathogen.

There are clinical implications for these data. For example, in more severely stressed individuals, the immune response could be sufficiently down-regulated to delay and/or inhibit the synthesis of adequate levels of protective antibodies and/or a T-cell response sufficient to provide adequate protection to a infectious agent. This may be particularly important in groups of individuals who are already at a high risk for infection due to alterations in the immune response. Two examples are the increased risk for morbidity/mortality in the elderly to influenza virus and pneumococcus infections, and the increased risk for secondary infections in AIDS patients (22, 23). In addition, since an individual is generally not monitored for an antibody response after receiving a vaccine, one may falsely assume that immunological defenses to an infec-

tious agent are operative. In the case of the HBsAg vaccine, where protection occurs over a 6-month period and a majority of individuals may not serovonvert until sometime between the second and third vaccine inoculation, some health workers who are at high risk for exposure to Hep B virus may not have sufficient immunity, particularly if the level of virus exposure is high. The data could also have implications for the military. Military personnel recently operating in the Persian Gulf were probably much more psychologically stressed than medical students taking examinations. All personnel would have received a battery of vaccinations, some primary and some booster. Since military personnel under conditions of war are at high risk for infectious disease, these data suggest that antibody screening to confirm protection in high risk groups would be worth considering. Future trials with other vaccines need to be conducted to determine the universality of these observations.

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