Psychosocial Modifiers of Immunocompetence in Medical Students

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This study addressed the effects of a naturally occurring stressor on components of the immune response. Blood was drawn twice from 75 first-year medical students, with a baseline sample taken one month before their final examinations and a stress sample drawn on the first day of final examinations. Median splits on scores from the Holmes–Rahe Social Readjustment Rating Scale and the UCLA Loneliness Scale produced a $2 \times 2 \times 2$ repeated measures ANOVA when combined with the trials variable. Natural killer (NK) cell activity declined significantly from the first to the second sample. High scorers on stressful life events and loneliness had significantly lower levels of NK activity. Total plasma IgA increased significantly from the first to second sample, while plasma IgG and IgM, C-reactive protein, and salivary IgA did not change significantly

INTRODUCTION

Data from a number of different laboratories suggest that stress produced in a variety of different ways affects immunocompetency (1–3). These alterations in immunity are thought to be the basis for increased susceptibility to infectious and malignant disease. For example, daily exposure to a shock avoidance task resulted in increased susceptibility to herpes simplex virus (HSV) (4), poliomyelitis (5), and polyomavirus infections (6) in mice. An example of an interaction between stress and virus infection is shown in a series of studies in which the level of stress was varied in mice that were inoculated with Coxsackie B-2 virus. Neither the stressful environment nor the inoculation of the virus was sufficient by itself to produce clinical disease; however, the combination resulted in a significant infection as measured by decreased body weight in inoculated mice (7).

Furthermore, experimentally induced stressors such as shock and crowding can modify the natural history of neoplasia. In a mouse spontaneous tumor model, 7% of mice raised in a protected environment had tumors at one year of age, compared with 92% of a group of mice that had been raised in a stressful environment (8). Inescapable shock also resulted in earlier tumor appearance and shortened the time between tumor implantation and death (9).

While a number of animal studies have demonstrated the immunosuppressive effects of stress, the number of human studies using psychosocial variables and/or repeated measures is very limited. Persons whose spouses had died had decreased thymus-derived (T) lymphocyte response to mitogen stimulation after

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bereavement when compared to nonbereaved controls (10). Such immunosuppression may contribute to the well-documented increase in morbidity and mortality associated with bereavement (11, 12). Other studies have shown that T-lymphocyte response to mitogen stimulation was also decreased by 48 hr of sleep deprivation, as was granulocyte function (13). Five days after the vigil all measures were back within predeprivation range.

The hypothesis that stressful life events or life changes may increase susceptibility to infection or exacerbate preexisting illness is widely accepted. However, there are a number of methodologic problems in the life events literature that frequently leave ample room for alternative explanations. Prospective studies using objective health indices are necessary to demonstrate the effects of stressful life changes on illness.

Recent interest in other moderator variables in the presumed stress–illness relationship has led to a rapid growth in social support research. Unfortunately, the social support literature has a number of shortcomings, the most important being inadequate conceptualization and operationalization. The study of loneliness is a closely related area having greater conceptual clarity and fewer measurement problems than social support (14). Unlike social support, research on the medical consequences of loneliness has been very limited thus far.

We were interested in the effects of a naturally occurring stressor on the immune response in a healthy adult population. Medical student subjects were bled twice, one month before final examinations and at the beginning of their final examination sequence. Stress-produced immunosuppression, if present, would be apparent by comparison of the baseline sample with the stress sample in our prospective design. Additional relationships of interest were the effects of stressful life events and loneliness on immunologic measures. An association between either parameter and immunologic variables would provide empirical support for their hypothesized roles as mediators of illness. Consistent with previous research, high scorers on either or both scales were expected to describe themselves as more distressed than low scorers on self-report measure. An association between high stressful life events and poorer academic performance was predicted, as has been described in previous studies (15).

As part of this study, we examined natural killer cell (NK) activity, one indicator of the cellular immune response. Natural killer cells are lymphoid immune cells that appear to have specific and preprogramed antitumor and antiviral activity. These cells have been shown to be of vital importance in preventing tumor development and spread (16). As such, they appear to form part of an antitumor surveillance system. Alterations in NK activity induced by stress would be of extreme interest, since this would suggest a pathway by which stress could increase the risk of malignant disease.

We also attempted to demonstrate quantifiable changes in several aspects of the humoral immune response, which could be associated with stress. These measures included total plasma IgG, IgA, IgM, C-reactive protein (CRP), and salivary IgA.

METHODS

Subjects and Timing of Samples.

Volunteers for research on stress and the immune response were solicited from the first-year medical

student class at The Ohio State University College of Medicine. The only inducement for participation was the promise of feedback on their immunologic data. Of the 175 students in the class, 26 females and 50 males agreed to participate. One man did not return for the second blood sample collection, leaving a total sample of 75 subjects. The average age of both male and female subjects was 23.

The initial blood sample was obtained 1 month after a major examination and one month before the series of final examinations for the first year were to begin. The second blood sample was obtained on the first day of the final exam week, after students had completed their first two examinations. Both were scheduled during the middle of the day.

Preparation of Lymphocytes and NK Assay

Blood specimens were drawn by peripheral venipuncture into heparinized glass tubes. Blood was diluted 1 : 2 with trypsin diluent (calcium and magnesium-free) and 10 ml was layered onto 4 ml Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway NJ). Samples were centrifuged at 450 × g for 30 min in an IEC tabletop centrifuge. Mononuclear cells were harvested from the interface and washed three times with RPMI 1640 medium to remove all Ficoll-Hypaque. Final cell pellets were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated pooled human serum (OSU Blood Bank), 2 mM L-glutamine, 24 mM Hepes, pH 7.21, 24 mM $NaHCO_3$, and 50 μg/ml gentamicin. Cell concentration was determined by Coulter counter and concentration adjusted by adding additional media.

Target cells were prepared using K562 cells from ongoing cell cultures. One million cells were washed and placed in 0.3 ml media with 75 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, MA). Target cells were pulsed for 1 hr, washed twice, then allowed to leach in media for 30 min. Cell concentrations were restandardized using trypan blue exclusion and a hemocytometer.

Lysis of target cells was determined in a 4-hr microtiter cytotoxicity assay. Triplicate aliquots (0.1 ml) of the target cell suspension were added to effector cell suspensions, to make a 10 : 1 effector to target cell ratio, and seeded into 96 well microtiter plates (Limbro, Hamden, CT). Additional wells containing only target cells in media or target cells in media containing 1% sodium dodecyl sulfate were used to determine spontaneous and maximal release of radioactivity, respectively. Plates were incubated for 4 hr in a 5% CO_2 incubator at 37°C.

Plates were centrifuged at 800g for 8 min. Supernates were harvested using a titertek supernate collection system (Flowlabs by Skatran, Norway), and activity was determined by the release of ^{51}Cr into the supernate.

Supernates were counted, using a Beckman 9000 gamma counter. Lysis was calculated by the formula

$$\frac{CPM_{sample} - CPM_{spontaneous}}{CPM_{maximum} - CPM_{spontaneous}} \times 100$$

Quantification of Plasma Immunoglobulins

The immunoglobulins were quantified by rate nephelometry as performed by the automated Beckman ICS (Beckman Instruments, Brea, CA 92621) (17). Plasma samples were placed in sample cups within a carousel tray. The instrument aspirated the sample, automatically diluted it (1 : 216 IgG; 1 : 36 IgA; 1 : 36 IgM, and neat secretory IgA) and injected the diluted sample into the reaction chamber. After a baseline nephelometric reading, the appropriate pretitered antiserum was injected into the chamber and the rate of immune complex formation was measured nephelometrically. This rate was compared to a standard curve previously determined using one calibrator. During the time of assay the coefficients of variation for IgG were 2.2%, IgA 2.3%, and IgM 2.2%.

Self-Report Measures and Grades

The Brief Symptom Inventory (BSI), a 53-item short form of the Symptom Checklist-90 (SCL-90) (18), provided data on nine primary symptom dimensions and three global distress indices. Each of the items was rated on a five-point scale from zero ("not at all") to four ("extremely") according to the amount of discomfort the problem caused in the last week. It was administered at the same time the blood samples were obtained.

The Social Readjustment Rating Scale (SRRS) (19) was administered at the time the first blood samples were obtained. It was used to assess life changes that had occurred within the last year. Weighted values were used to provide a sum of life changes; these were divided at the median, 140.5, to form high ($\bar{X}$ = 235.13) and low ($\bar{X}$ = 89.22) stressful life events groups.

The UCLA Loneliness scale (20) was included during the first data collection to provide a brief subjective measure of the adequacy of interpersonal contacts. Scores were divided at the median, 34.5. The mean of the high loneliness group was 41.62, and the mean of the low loneliness group was 28.6.

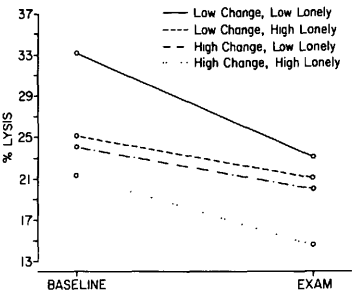

Fig. 1. Changes in NK activity from the baseline sample to the examination sample, using a 10 : 1 effector to target cell ratio.

RESULTS

Immunologic and self-report data were analyzed using a 2 × 2 × 2 repeated measures analysis of variance design. There were two between-subjects variables, life stress and loneliness, and one within-subjects variable, trials. No significant effects were found when sex was included as an additional variable in preliminary analyses with the exception of plasma IgA; therefore, data were combined for subsequent analyses (except IgA).

Measurement of NK Activity

Natural killer cell activity was measured in both medical student blood samples. Using an effector to target cell ratio of 10 : 1, we found that there was a significant decrease in NK activity from the first to the second blood sample, $F\,(1, 68) = 9.87, p < 0.003$. These data are shown in Figure 1. There were also main effects for stressful life events, $F\,(1, 68) = 8.19, p < 0.006$, with high scorers having lower NK activities. Similarly, the high loneliness scorers had lower levels of NK activity than low scorers, $F\,(1, 68) = 5.48, p < 0.02$.

Plasma Immunoglobulins, Salivary IgA, and CRP

Because of technical difficulties, plasma from the second sample was available for 44 subjects for total plasma IgA and IgM analyses, and for 37 subjects for analysis of plasma IgG. These data were not distributed evenly across groups, i.e., data for 78% of the high loneliness, low stressful life events groups were unavailable. Therefore, loneliness and stressful life events were not included as variables in these analyses.

All three of the plasma immunoglobulins increased from the first to the second sample, but the increase only reached significance for IgA, $F\,(1, 42) = 6.05, p < 0.02$. Females had lower levels on both blood samples than males, $F\,(1, 42) = 6.39$, $p < 0.02$, and there was a significant interaction between sex of subject and change over trials, $F\,(1, 42) = 21.52, p < 0.001$. The mean plasma IgA levels for males increased from 177.09 to 258.83 mg/dl, while females had a smaller increase, from a mean of 148.33 to 173.42 mg/dl. The increases for IgM, from 162.95 to 188.43

mg/dl, (F (1, 42) = 2.61) and IgG, from 1261.44 to 1404.61 mg/dl, (F (1, 35) = 1.43) did not reach significance.

Sixty-five of the subjects provided sufficient saliva on both samples for salivary IgA analyses. There was not a significant change from the first to the second trial (F (1, 57) = 0.05), nor were there significant effects for sex, loneliness, or stressful life events.

Four subjects out of 75 had higher than normal CRP levels on the baseline sample, compared to 4 out of 53 subjects in the second sample. Statistical analyses were not performed because of the very small number of deviant samples.

Self-Report Data and Grades

The BSI data indicated that greater distress was reported during final examinations than had been reported at baseline. These data are shown in Table 1.

Significant interactions between stressful life events and trials occurred on three BSI scales, the General Symptom Index, F (1, 71) = 3.84, $p < 0.05$, paranoia, F (1, 71) = 4.64, $p < 0.03$, and psychoticism, F (1, 71) = 5.03, $p < 0.03$. These interactions were the result of larger increases by high stressful life event scorers; e.g., the mean GSI T-score for low scorers went from 52.43 to 53.25, while high scorers had a baseline mean of 51.10 and an examination day mean of 56.18. There were no significant main effects or interactions as a function of loneliness.

TABLE 1. Changes in Mean BSI *T*-Scores from the Baseline Sample to the Examination Sample

BSI Scale	Baseline	Examination
Somatization[a]	49.17	52.70
Obsessive–Compulsive symptoms[b]	58.84	63.84
Interpersonal sensitivity	58.53	57.61
Depression[c]	57.27	59.14
Anxiety[b]	55.89	67.19
Hostility[b]	52.23	58.30
Phobic anxiety	53.92	54 85
Paranoia	52.40	52.75
Psychoticism	57 27	59.41
General symptom index[d]	52.49	54.89
Positive symptom total	57.13	60.41
Positive symptom index	54.30	58.47

[a] $p < 0.001$
[b] $p < 0.0001$
[c] $p < 0.05$
[d] $p < 0$ 01

To help rule out the possibility that the self-ratings and/or immunologic data were the result of preexisting health problems, students were asked during the second bleed if they had any significant health problems, including allergies. Eleven students listed allergies, and they were reasonably evenly distributed across the four criterion groups. No other problems were listed that might have affected the immunologic data.

The grades of research participants were compared with those of fellow classmates to determine whether our self-selected sample could be potentially biased, e.g., poorer students might have volunteered disproportionately because of concerns about the effects of academic stress. Although there was a trend for the research participants to have somewhat higher grades than nonparticipants, there was not a significant difference on grades before the final, F (1, 174) = 2.26, nor was there a significant difference on the final examination, F (1, 174) = 1.84.

The grades of participants were then analyzed using the life change and loneliness dimensions. Loneliness did not have any significant effects. Stressful life events had a significant impact, with high scorers having lower average exam scores before

the final, $F\ (1, 71) = 6.93, p < 0.01$, as well as on the final, $F\ (1, 71) = 5.13, p < 0.03$. The difference in means for both tests was between 3 and 4 points on a 100 point scale.

DISCUSSION

We examined the effects of a relatively mild stressor on several immunologic parameters, including NK activity, and total levels of plasma IgA, IgG, IgM, CRP, and salivary IgA in a prospective design. The NK cell data provide direct evidence of immunosuppression associated with increased distress in a young and otherwise healthy population. Since this relationship was found in medical students who had previously distinguished themselves by their performance on examinations, the finding of decreased NK activity in such a sample is particularly striking. It appears that the immune system is sensitive to milder stressors than those sampled previously, e.g., bereavement and 48 hr of noise and sleep deprivation.

Both stressful life events and loneliness appeared to be associated with NK cell function. The significant association between stressful life events and NK activity lends greater credence to the position that an accumulation of stressful life events can have negative consequences on health. The problem of separating illness from treatment-seeking behavior, frequently occurring in life events research, was avoided by our use of immunologic data in a prospective design (21).

There was a significant relationship between loneliness and NK activity, but loneliness did not have an effect on self-reported distress. In previous studies, substantial relationships have been found between loneliness and depression and anxiety (20). Such an association with more pervasive distress might have provided one route for possible effects on health, since the immune system appears reponsive to distress. However, the absence of such effects in our BSI data suggests that there may be dimensions of loneliness independent of distress that have significant implications for health. Moreover, it is interesting to speculate that the immunosuppression associated with bereavement (10) may be in part a function of the loneliness that accompanies the loss of a loved one.

The increase in plasma IgA observed in this study is surprising, in that even more extreme stressors in past studies have failed to show changes in the humoral immunologic component. However, in previous research on psychosocial factors and immunoglobulins, IgA levels have been significantly more elevated among breast cancer patients who suppressed anger than among others (22). The half-life of plasma IgA is 6–8 days, compared to 25–35 days for plasma IgG, and 9–11 days for plasma IgM (23). Therefore, of the three plasma immunologlobulin measures studied, IgA would be the best candidate for the demonstration of reactivity to examination stress, given the timing of samples. Moreover, the duration of the stressor and the measurement time may have had an impact on the plasma IgA results. In previous studies, the stressors have frequently been relatively brief and time limited, e.g., 48 hr of sleep deprivation (13). Preparations for final examinations and associated concerns about performance commonly begin at least a week in advance of examinations. The discrepancy between plasma and salivary IgA data is likely to be a result of technical differences. Salivary IgA levels are dependent on flow rate, so that inadequate hydration can have an impact

on measured levels, and we did not have flow rate data available for comparisons.

Our data demonstrate the effects of a relatively minor stressor, final examinations, on an important component of the immune response, NK activity. We were also able to show that two variables thought to have an impact on illness, stressful live events and loneliness, did indeed have significant effects on NK activity. Any statement relating these variables and decreased NK cell function to malignant disease would be speculative, however, and will require more knowledge about the function of NK cells. Unfortunately, we do not have data on any changes in the use of drugs or alcohol, amount of sleep, or nutritional status in relation to the baseline and final examination weeks. However, if stress can be shown to have an impact on the antiviral activity of NK cells (16), it would explain the popular belief that viral illness is more likely to follow stressful periods, as well as provide immunologic support for previous research linking stressful life events and some upper respiratory illnesses.

SUMMARY

We examined the effects of examination stress on several immunologic parameters including natural killer (NK) cell activity, total levels of plasma IgA, IgG, and IgM, salivary IgA, and C-reactive protein (CRP). Blood was drawn twice from 75 first-year year medical students, with a baseline sample taken one month before their final examinations. Median splits on scores from the Holmes-Rahe Social Readjustment Rating Scale and the UCLA Loneliness Scale produced a $2 \times 2 \times 2$ repeated measures ANOVA when combined with the trials variable. Natural killer cell activity declined significantly from the first to the second sample. Both stressful life events and loneliness had significant effects on NK activity in the expected direction, with high scorers on either having lower NK cell levels. Plasma IgA increased significantly from the first to the second sample, while plasma IgG and IgM, salivary IgA, and CRP did not change significantly.

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