Urinary Cortisol Levels, Cellular Immunocompetency, and Loneliness in Psychiatric Inpatients

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This study examined the associations among loneliness. stressful life events. urinan cortisol levels. and immunocompetency. Blood and urine were obtained from 33 psychiatric inpatients on the day after admission, at which time the patients completed the UCLA Loneliness Scale, the Psychiatric Epidemiology Research Interview Life Events Scale (PERI), and the MMPI. Patients who scored above the median on loneliness had significantly higher urinary cortisol levels. The high loneliness group also had significantly lower levels of natural killer cell activity. as well as a poorer T-lymphocyte response to phytohemagglutinin. The high loneliness subjects described themselves as more distressed than the low loneliness group on the MMPI. There Were no consistent significant effects on either the immunologic measures or the MMPI associated with the PERI.

INTRODUCTION

There is growing support for the view that psychosocial variables can have an effect on the immune system, presumably through the mediation of the central nervous system (1, 2). Although there are only a limited number of studies using human subjects, the data suggest that acute stressors can have immunosuppressive effects, particularly on the cellular immune response. For example, Bartrop et al. (3) found that bereaved spouses had decreased T-lymphocyte response to mitogen stimulation, in comparison to nonbereaved controls. In other research the responsivity of T-lymphocytes to phytohemagglutinin (PHA) was decreased in astronauts on the stressful splashdown day, but showed a rapid return to preflight levels (4). Using a prospective design. Kasl et al. (5) found an increased risk of infection by Epstein-Barr Virus (EB\') in military cadets who had high levels of motivation. a poor academic performance. and "overachieving" fathers.

In a recent study, we found that natural killer cell (NK) activity declined significantly in blood samples from medical students taken before and during examinations. NK activity is thought to have a key role in tumor surveillance, as well as in the control of viral infections (6). Two other factors were significantly related to NK activity in our sample. stressful life events and loneliness, with high scorers on both having lower NK activity (7).

High loneliness scorers among the medical students also had signifcantly higher EBV antibody titers than low scorers (8). This was noteworthy because of the immunologic mechanisms involved in the control of the EBV. After the initial active EBV infection, the virus genome is repressed in a latent state within host B-lymphocytes. It is thought that cellular immunity is important in the regulation of latent infections associated with EBV and other herpes viruses (9).

We pursued the linkage between EBV and loneliness further, since the higher antibody titers found in the lonelier medical students were presumably reflecting poorer cellular immune competence (10). Blood was obtained from medical students before and during final examinations, and on their return from summer vacation in order to study cellular transformation by EBV. There were significant changes in the transformation of B-lymphocytes by EBV in mixed cultures of T- and b-lymphocytes, with the lowest transformation levels and the highest self-ratings of distress coinciding on the final examination bleed. There was again a significant effect for loneliness, with high loneliness subjects having generally lower transformation levels (11). The implications of these data are discussed in reference to the cellular immune response.

The pervasive effects of loneliness on these immunological measures led to the current study on urinary cortisol levels, cellular immunocompetence, and loneliness in a psychiatric population. We were interested in attempting to reproduce and extend the previous finding of decreased immunocompetence associated with higher levels of loneliness and stressful life events. Psychiatric inpatients were used because as a group they have smaller social support networks (12) and they describe themselves as significantly lonelier than nonpsychiatric controls (13).

Urinary cortisol levels were measured in this group because there are data suggesting that isolation may have an impact on the endocrine system, particularly on corticosteroid levels. While there appear to be homeostatic controls associated with hormonal and immunological systems during chronic physical stressors, physiologic adaptation to social stressors may not readily occur (14). It has been shown that isolation can produce increased corticosteroid levels, and the elevations can persist after chronic isolation has ended (15). Such corticosteroid elevations may have immunologic consequences; in vivo treatment of mice and humans with adrenocortical hormones can produce significant transient reductions in NK activity (6). Corticostero(ds can also inhibit T-lymphocyte function (16).

Urinary cortisol levels provided one measure of adrenocortical activity in this study. Immunologic measures included NK activity and reactivity of lymphocytes to phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Pokeweed mitogen stimulates both T- and B-lymphocyte proliferation in vitro, while PHA stimulates only the former.

METHOD

Subjects

Newly admitted nonpsychotic psychiatric inpatients al the Ohio State University Hospital were used as subjects in the research. Mentally retarded patients. those with any physical problems that might have had a significant immunologic component. and those with any evidence of recent alcohol or drug abuse were excluded From the study. The final sample consisted of 21 women and 12 men, with an age range of 18-52 and a mean age of 34.0. Subjects completed a brief interview and the questionnaires. and allowed additional blood to be drawn at the time of the routine I admission blood draw.

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Self-Report Measures

The UCLA Loneliness Scale was used to provide a brief subjective measure of the adequacy of interpersonal relationships (13). The original validational research for the UCLA scale was based on a multitrait-multimethod model. Multiple regression analyses revealed that the loneliness index explained significant amounts of variance beyond the amount accounted for by distress and personality measures. Also, after statistically controlling for the effects of mood and personality variables, loneliness scores were significantly related to relevant interpersonal contact indices (13).

The distribution of scores was divided at the median, 48.5, to provide high (mean = 57.89) and low scoring groups (mean = 40.88). Higher scores indicate self-assessed loneliness.

The Minnesota Multiphasic Personality Inventory (MMPI) is routinely administered to all new admissions in the psychiatric division of University Hospital. These data provided a well-validated measure of distress for this population.

The Psychiatric Epidemiology Research Interview Life Events Scale (PERI) was used to assess potentially stressful recent life changes (17). Weighted scores were divided at the median. 3000. The high scoring groups (mean = 40.88). Higher scores indicate greater self-assessed loneliness.

Urinary Cortisol

Urinary cortisol was measured by a competitive protein assay as described by Murphy (18). Briefly, 1 m) of urine was extracted with methylene dichloride and added to human plasma. One milliliter of Vitiated corticosterone (New England Nuclear) was added and the amount of radioactive binding was determined using a scintillation counter. The human plasma was standardized for competitive binding globulins. Cortisol levels were normalized against creatinine (19) to wg/g creatinine, so we could use random urine samples instead of 24-hr collections.

NK Assay. NK activity was determined by a microtiter "Chromium release cytotoxicity method. Briefly, mononuclear cell populations were obtained by Ficoll-Hypaque separation of diluted whole blood. After centrifugation and multiple washings to remove all adherent Ficoll-Hypaque., the cells were resuspended in a complete medium consisting of RPM1 1640 supplemented with 10% heat inactivated human serum (OSU Blood Bank), 2 mM L-glutamine (Gibco, Grand Island, NY), 25 mM Hepes pH 7.2 (Sig Ms. St. Louis, MO), 24 mM NaHCO3, and 50,ug/ml gentamicin (Schering Corp., Kenilworth, N)). A viability cell count was performed using trypan blue exclusion with a hemocytometer and medium was added to make the lymphocyte concentration 2 x 10^0 cells mL. The target cells used in the assay were K562 cells. a myeloid cell line. One million K562 cells were labeled with 75 uCi $Na2s^1CrO4$ (New England Nuclear) in 0.2 ml medium for 1 hr. Cells were then washed twice, leached for 30 min, and counted.

Triplicate (0.1 ml) aliquots of lymphocyte and labeled target cell suspensions were placed in wells of 96-well plates (Limbro, CN). Effector to target cell ratios of 40: 1, 20: 1, and 10: 1 were obtained by successive dilutions of the original lymphocyte suspension. These determinations gave us our experimental lysis values. In addition, triplicate wells with target cells and media only, and target cells and detergent (1% sodium dodecyl sulfate) were prepared :n order to determine spontaneously released radioactivity and maximal lysis. All plates were centrifuged at 300g for 3 min and incubated for 4 hr in a 5% C02 incubator at 37°C.

Plates were then centrifuged at 600g for 5 min and the supernates harvested using a Titertec harvesting frame system (Skatron, Norway). Supernates were counted in a Beckman 9000 gamma counter. Results are reported as percent lysis using the formula:

$$\frac{\text{experimental CPM - spontaneous release CPM}}{\text{maximal CPM - spontaneous release CPM}}$$

Spontaneous release was less then 5% of maximal release in all cases.

Blaslogenesis. Mitogens were used at a final concentration of 2.5, 5.0 and 10.0 jag/ml for PWM and 0.25, 0.5, 1.0 and 2.0;Lg/ml for PHA. Each assay was performed in triplicate. Complete medium was used for baseline controls. One-tenth of a milliliter of mitogen was added to 1 x 10^6 lymphocytes (in 0.1 ml medium) in 96 well plates and incubated at 37°C for 48 hr. Fifty microliters of tritiated thymidine (10 pCilml, specific activity 82 Ci/mM) were added to each well and the plates incubated at 37°C for 4 hr. Cells were harvested onto GFIA filters. Radioactivity was measured using a Beckman LS7000 scintillation counter.

RESULTS

The data were analyzed using an analysis of variance design, with two between subjects variables, loneliness and stressful life events. The initial analysis also included sex as a variable. However, there were no differential sex of subject effects with respect to age, loneliness, stressful life events, urinary cortisol levels, immunologic competence, or distress on any of the MMPI scales, with the exception of MMPI scale 5, masculinity-femininity; therefore, sex was not used as a variable in subsequent analyses. The majority of the patients received adjustment disorder, dysthymic disorder, or anxiety disorder diagnoses. There were no significant differences across the four groups in the incidence of the various relative diagnostic categories.

Self-Report Data

The MMPI scale T-scores are shown in Table 1, along with significance values. Although the high stressful life event subjects had higher scores on most scales, this difference did not reach statistical significance on any scale.

TABLE 1. Mean MMPI T-Scores

MMPI Scale	Low Loneliness	High Loneliness
L	53.38	52.71
F'	62.69	73.76
K	48.13	51.71
1 (Hypochondriasis)	63.00	64.41
2 (Depression)b	74.69	83.88
3 (Hysteria)	69.13	69.82
4 (Psychopathic Deviate) '	74.94	82.65
5 (Masculinity-Femininity)	52.06	53.00
6 (Paranoia)b	69.75	76.94
7 (Psychasthenia)'	69.38	80.18
8 (Schizophrenia)b	71.50	84-06
9 (Hypomania)	63.50	61.94
0 (Social Introversion)b	59.75	68.29

[a]p < 0.01
[b]y < 0.05

Urinary Cortisol

The high loneliness inpatients had significantly higher levels of urinary cortisol than low loneliness subjects, F (1, 29) = 4.22, p <0.05. The mean level of the high loneliness group was 30.77 Kg/g creatinine, in contrast to the low loneliness: group's mean of 16.56)Ag/g creatinine There were no significant effects associated with stressful life events.

NK Activity

There were significant differences between the high and low loneliness for all three NK target to effector cell ratios, as shown in Figure 1. The significance levels for loneliness across the three ratios were F (1, 25) = 4.88, p <0.04 for the 40: 1 ratio F(1, 25) = 4.07, p <0.05 for the 20: 1 ratio and F(1, 25) = 4.01, p<0.05 for the 10: 1 ratio. Data on NK activity were not available on four subjects due to technical problems. There were no significant effects as function of stressful life events, or as a

function of the interaction between stressful life events and loneliness.

Blastogenesis

The high loneliness group had a poorer response to PHA stimulation, with significant differences at concentrations of 0.25 µg/ml (F1, 29) = 4.40, p <0.04), and 0.50 µg/ml (F(1, 29) = 5.03,p <0.03), and a near significant difference at a concentration of 1.0 µg/ml (F(1, 29) = 2.27, p <0.14). The difference was not significant for the 2.0 µg/ml concentration (F(1, 29) = 0.31). These data are shown in Figure 2.

There were no consistent significant effects in the subjects' response to PWM stimulation as a function of the independent variables. Although the interaction between stressful life events was significant for the *5.0* µg/ml concentration (F(1, *29) = 6.21, p <0.02),* this interaction was not significant for either the *2.5* pg/ml concentration (F(1, *29) = 0.70)* or the 10.0)Ag/ml concentration (F(1, *29) = 1.18).* The logarithmic means for the *5.0* ug/ml concentration were *0.75* for the low stress, low lonely group; *1.07* for the high stress, low lonely group; *1.14* for the low stress, high lonely group; and *0.82* for the high stress, high lonely group.

In order to evaluate the relative contributions of loneliness and distress to immunocompetency, multiple regression analyses were performed. For all three *NK* effector to target cell ratios and for all but two of the PHA dilutions, loneliness emerged as the best predictor of immunocompetence. Correlations among loneliness, MMPI scales, and the immunologic variables were low, however.

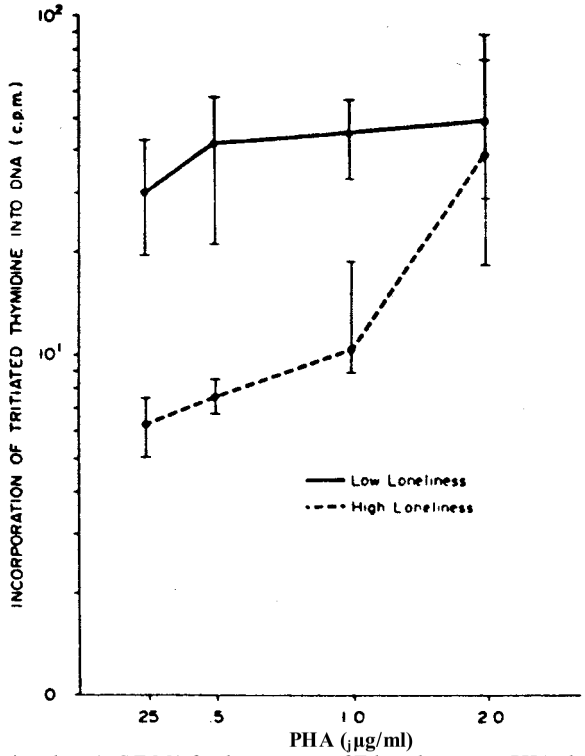

Fig. 2. Mean logrithmic values (= S.E.M.) for the response of T-lymphocytes to PHA obtained from psychiatric inpatients.

DISCUSSION

In this study we found a significant association between two components of the cellular immune response and loneliness, with high loneliness subjects having significantly lower NK activity as well as a poorer T-lymphocyte response to PHA, when compared to the low loneliness group. High loneliness subjects also had significantly higher urinary cortisol levels. The association between *NK* activity and loneliness followed the same pattern as was found previously in medical student subjects (7). The PHA data are consistent with other research on the responsiveness of T-lymphocytes to various stressors, as discussed earlier (3, 4), and provide further support for the interplay between psychosocial variables and the immune response.

The absence of any consistent significant association between the independent variables and the response of mixed T- and B-lymphocytes to PWM is not surprising. Although some researchers see cellular immunity as more responsive to various acute physical stressors than humoral immunity (20), the effects of stress on the humoral response have been documented in humans and animals (21). It is possible that the differences observed between the high and low loneliness groups in their T lymphocyte response to PHA were not vigorous enough to be detected in the mixed cell population used.

There are several possible explanations for the absence of consistent effects for stressful life events in both the immunologic and self-report data. The extremely high levels of distress that frequently precipitate psychiatric admissions maybe of sufficient intensity to overpower more subtle distress-related indices. Also, the psychiatric population is far more heterogeneous with respect to socioeconomic and demographic variables than the medical student population in which significant effects for stressful life events were previously found. This variability may have overshadowed the effects of stressful life changes (22).

Although the effects of interpersonal support on health-related outcomes have received attention in the related social support literature, the association between loneliness and health has not been comparably addressed (23). The social support "buffering" hypothesis suggests that the effects of support on stress are interactive, with support presumably having little effect on health-related outcomes when stress is low; the protective effects of support are thought to be maximized during periods of high stress (24). Extrapolating from the buffering hypothesis to our loneliness data, it is possible that the relatively high levels of distress which both the inpatients and the previous medical student sample (7) were experiencing may have influenced the outcomes. It would be interesting to see if loneliness is significantly related to urinary cortisol levels and cell-mediated immunity in less distressed populations, or, alternatively, if persistent corticosteroid elevations and decreased cellular immunocompetency can be found

across populations that differ in the frequency and intensity of their social contacts.

One factor that may contribute to the higher urinary cortisol levels and poorer immuncompetence of the high loneliness group is their higher level of distress. However, the high loneliness medical student subjects in our previous research did not describe themselves as significantly more distressed than low loneliness subjects, suggesting that there may be other pathways through which loneliness influences immunocompetence.

The demonstration of a significant association between loneliness and cellular immunocompetency in two very different populations, psychiatric inpatients and medical students, provides evidence for the impact of loneliness on health. The high loneliness inpatients were significantly more distressed than the low loneliness group, and their distress may have provided one pathway for the elevated urinary cortisol levels and decreased immunocompetency. This decreased immunocornpetency may have an effect on health; the mortality rate among psychiatric patients is one and one-half to two times as high as among nonpatients even when the high-risk chronically ill, aged, and alcoholic subpopulations are removed from consideration (25). Furthermore, it is possible that the decreased T-lymphocyte function previously found with bereaved spouses (3) may be reflecting the effects of a sudden increase in acute or severe loneliness, as well as more general distress. This group also has morbidity and mortality well in excess of comparable controls (26, 27). Loneliness appears to be an important variable for consideration in future research on stress and immunocompetency.

SUMMARY

This study examined the associations among loneliness, stressful life events, urinary cortisol levels, and immunocompetency. Blood and urine were obtained from 33 psychiatric inpatients on the day after admission, at which time they completed the UCLA Loneliness Scale, the Psychiatric Epidemiology Research Interview Life Events Scale (PERI), and the MMPl. Patients who scored above the median on loneliness had significantly higher urinarv cortisol levels. The high loneliness group had significantly lower levels of natural killer cell activity, as well as a significantly poorer T-lymphocyte response to phytohemagglutinin. The high loneliness subjects described themselves as significantly more distressed than the low loneliness group on the *MMPI*. There were no consistent effects associated with either loneliness or stressful life events in the response of T- and B-lymphocytes to pokeweed mitogen. There were no consistent significant effects on either the immunologic measures or the *MMPI* associated with the PERT. The importance of loneliness as a potential modifier of health-related outcomes was discussed.

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