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Distress and DNA Repair in Human Lymphocytes

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This research assessed differences in DNA repair in lymphocytes from high- and low-distressed individuals. A median split on Minnesota Multiphasic Personality Inventory (MMPI) Scale 2 divided 28 newly admitted nonpsychotic psychiatric inpatients into high- and low-distress subgroups. The high-distress subgroup had significantly poorer DNA repair in lymphocytes exposed to X-irradiation than low-distress subjects. We also found that lymphocytes obtained from this psychiatric sample had significantly poorer DNA repair than lymphocytes from nonpsychiatric control subjects when compared 5 hr after X-irradiation. A high level of distress therefore appears to be associated with significant dysfunctional differences at the molecular level which may have important implications for health. These data provide evidence for a direct pathway through which distress could influence the incidence of cancer.

KEY WORDS: distress; DNA repair; psychoimmunology; stress.

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INTRODUCTION

There is growing evidence that various stressors may affect the development and course of infectious and malignant disease through their impact on the immune response (Ader, 1980). While such effects have been demonstrated in a number of animal studies (Monjan, 1981; Laudenslager *et al.*, 1983), the human literature is more limited (Bartrop *et al.*, 1977; Palmbald, 1981; Jemmott *et al.*, 1983). In earlier studies, we found that medical students had significantly poorer natural killer-cell (NK) activity in blood samples obtained during examinations, in contrast to baseline samples drawn 1 month previously (Kiecolt-Glaser *et al.*, 1984a). Two other measures of the cellular immune response were also affected, the transformation of B lymphocytes by Epstein–Barr virus (EBV) (Kiecolt-Glaser *et al.*, 1984c) and antibody titers to three different latent herpesviruses (Glaser *et al.*, 1985).

In an attempt to explore further the links between distress and health-related changes, we assessed differences in DNA repair in lymphocytes obtained from high- and low-distressed individuals. Most carcinogens appear to induce cancer by damaging the DNA in cells, thereby producing mutant cells (Miller, 1978). The body's ability to repair damaged DNA is therefore particularly critical, since there is good evidence that faulty DNA repair is associated with a significantly increased incidence of cancer (Setlow, 1978; Takabe *et al.*, 1983). Faulty repair of damaged DNA can also result in important alterations in cell growth, cell division, and gene expression, as well as cell death (Hart *et al.*, 1978).

For this study we adopted the method proposed by Cook and Brazell (1976) to measure DNA repair by following the restoration of nucleoid sedimentation after irradiation. This method is particularly sensitive to DNA alterations such as strand breaks which reduce the migration or sedimentation rate by altering the three-dimensional structure of the molecule. When the DNA damage has been fully repaired, migration rates return to preir-radiation baselines. Such measurements provide an assessment of the restitution of the primary and secondary DNA structures, as well as higher-order structures which are more directly correlated with alterations in cellular physiology (Lipetz *et al.*, 1982a).

Comparisons of the extent of cellular DNA repair were initially made within a psychiatric sample divided into high- and low-distress subgroups. Psychiatric patients were used in order to maximize the level of distress studied in this initial investigation, since it was unclear whether there might be distress-related differences in DNA repair. Additional comparisons were also made between this psychiatric sample and age- and sex-matched Red Cross blood donors.

METHODS

Newly admitted nonmedicated nonpsychotic psychiatric inpatients were used as subjects because of the high levels of distress which are normally associated with psychiatric admission. Mentally retarded patients, those with any immunologic or hormonal dysfunctions, and those with any evidence of alcohol or drug abuse were excluded. The 28 subjects (18 women and 10 men) had a mean age of 32 years and a range from 19 to 59 years.

Subjects completed the Minnesota Multiphasic Personality Inventory (MMPI) on the first weekday after admission. Scale 2, depression, served as the distress measure. This scale provides an excellent, well-validated index of a subject's general level of distress or discomfort (Webb *et al.*, 1981). Subjects were divided into two groups of 14 using the *T*-score median of 80.5 to form high (mean = 91.3)- and low (mean = 67.5)-scoring distress groups. The overall group mean was 80.14, i.e., the mean distress score of this group was three standard deviations above the population mean and fell within the upper 1% of the adult population.

DNA Repair Assay

DNA repair was measured by the recovery of nucleoid sedimentation following irradiation with 100 rads of X-irradiation. Mixed peripheral leukocytes (MPL) were isolated from whole blood on standard Ficoll–Hypaque gradients. The MPL, composed primarily of lymphocytes, were resuspended at a concentration of 1×10^6 cells/ml and irradiated on ice with a Profex-ray unit calibrated at 200 rads/min (100 kV, 6 mA). The cells were pelleted, then resuspended in cold phosphate-buffered saline (PBS) immediately and layered onto gradients (time = 0) or resuspended in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, incubated at 37°C for 2 or 5 hr, and then prepared in PBS and layered onto gradients. Neutral sucrose gradients [15–30% (w/v) 1.95 *M* NaCl, 10 m*M* Tris-HCl (pH 8.0), 10 m*M* EDTA] were prepared in 1/16 × 2 3/8-in. polyalomer tubes (Beckman Instruments) to a volume of 3.6 ml/gradient. The nucleoids were prepared as previously reported (Lipetz *et al.*, 1982b). A 50-μl suspension of cells in PBS was added to 150 μl of lysis solution [2.6 *M* NaCl, 13 m*M* Tris-HCl (pH 8.0), 13.3 m*M* EDTA, 0.13% Triton X-100] which had been carefully layered onto the gradients. Lysis was allowed to proceed for 15 min in a mounted SW27 rotor with adaptors for accommodating two tubes per bucket (12 tubes per rotor). Following lysis, the gradients were centrifuged at 17,000 rpm for 70 min at 20°C. Gradients were fractionated from the top

using a meniscus following pump (Buchler). The gradient was pumped (2.5 ml/min) parallel with a 300 ng/ml 4′,6-diamidino-2 phenylindol (DAPI) solution (7.5 ml/min). The DAPI and gradient were mixed and passed through a flow fluorimeter (excitation, < 400; American Research Products Corp.). The DNA–DAPI signal was graphed on a strip chart recorder (ISCO). The locations of peaks in the gradients were calculated from the chart record.

Prior to determining the percentage ratios, the standard error was calculated for each control and each X-irradiated sample. The means of the standard errors ranged from 0.8 to 2.2, a range which is consistent with a low variability within samples.

The DNA repair values at 0, 2, and 5 hr represent the comparison of the migration of nucleoids from X-irradiated lymphocytes in a neutral sucrose gradient and the nucleoid sedimentation rates (NSR) of nucleoids from nonirradiated cells from the same person. A value of 100% would mean that the degree of repair (migration rate) after X-irradiation was equivalent to the nonirradiated control value. Values of less than 100% indicate incomplete repair.

RESULTS

Psychiatric-Sample Characteristics

Comparisons were made between the high- and the low-distress psychiatric inpatient groups to assess the possibility that the groups might differ systematically as a function of psychiatric diagnosis, sociodemographic variables, or cancer-related risk factors. These data are presented below.

The discharge diagnoses of the psychiatric patients are shown in Table I. There are no apparent differences between the psychiatric subgroups. Less than a third of the patients were given secondary (Axis II) diagnoses, and these did not appear to follow any particular pattern.

Sociodemographic data from the high- and low-distress psychiatric subgroups are shown in Table II. There were no significant differences as a func-

Table I. DSM-III Psychiatric Diagnoses, Axis I

Low distress	High distress
7 major depression	8 major depression
3 adjustment disorder	2 adjustment disorder
1 atypical depression	1 atypical depression
1 panic disorder	1 panic disorder
1 dependent personality	1 dysthymic disorder
1 atypical eating disorder	1 posttraumatic stress disorder

Table II. Sociodemographic Means and Standard Errors of Low- and High-Distress Psychiatric Inpatients

	Low distress		High distress	
Sex				
Males	4		6	
Females	10		8	
Age	32.07	(3.07)	35.08	(2.52)
Education (years)	13.14	(0.82)	12.23	(0.51)
Marital status				
Married	7		5	
Single	5		5	
Divorced/separated	2		4	
Occupation				
Major and lesser professionals, executives	2		0	
Administrative personnel, clerical, sales, farmers	1		3	
Skilled and semiskilled employees	1		3	
Unskilled employees, unemployed	4		5	
Housewives	3		1	
College students	3		2	

tion of sex, age, education, or marital status, and there were no clusters of subjects within any particular occupation.

Additional comparisons were made on variables associated with increased cancer risk and alcohol and tobacco use. The average alcohol intake per week (in 12-oz beer equivalents) did not differ significantly between groups. The mean was 6.14 in the low-distress group and 2.23 in the high-distress group [$F(1,26) = 1.55$]. The mean number of cigarette packs per week in the low-distress group (5.71) did not differ significantly from that in the high-distress group (7.38) ($F < 1$); half the patients in each group were smokers. The number of years the patients had smoked did not differ reliably ($F < 1$), with a mean of 8.86 in the low-distress groups and a mean of 7.46 in the high-distress group.

Comparisons on distress-related factors which might have physiological and/or diagnostic implications (weight loss, sleep disturbance, number of previous psychiatric admissions, and length of admission) also failed to differentiate between groups. No subject fell below the normal weight range for his or her height, and weight loss in the 2 weeks before admission did not differentiate between the two groups [$F(1,26) = 1.16$]. The mean loss for the low-distress group was 1.21 lb, in contrast to 0.31 lb in the high-distress group. Seven of the low-distress patients reported some sleep disturbance prior to admission, in contrast to eight of the high-distress patients. The mean number of previous admissions did not suggest that the high-distress group

Table III. MMPI *K*-Corrected *T*-Score Means and Standard Errors

MMPI scale	Low distress		High distress	
1 (hypochondriasis)**	61.42	(2.91)	77.57	(3.58)
4 (psychopathic deviate)**	66.42	(3.51)	81.50	(3.55)
7 (psychasthenia)**	66.42	(3.05)	80.78	(2.68)
8 (schizophrenia)*	67.08	(5.57)	82.57	(4.93)
0 (social introversion)*	56.75	(3.73)	71.14	(3.96)

*$P < 0.05$.
**$P < 0.01$.

was a more chronic sample ($\bar{X} = 1.00$) than the low-distress group ($\bar{X} = 0.57$) [$F(1,26) = 1.13$], nor did the length of hospitalization differentiate between groups ($F < 1$).

One final between-groups comparison was made to assess a possible risk factor associated with differences in DNA repair, blood pressure (Pero *et al.*, 1976). The psychiatric subgroups did not differ reliably on either systolic or diastolic blood pressure (both F's < 1); however, only two subjects fell within the low end of the borderline range of hypertension and none were hypertensive.

Comparisons were made between the psychiatric subgroups on the three standard MMPI validity scales, as well as the nine standard profile scales, excluding depression. While there were no significant differences on the three validity scales, there were significant differences on five of the standard profile scales. These differences are shown in Table III and support the suggestion that the high-distress group was a more distressed group across symptom dimensions, rather than simply more depressed.

DNA Repair

The DNA repair data were analyzed using a repeated-measures analysis of variance. This design allowed us to assess (1) the differences in DNA repair between the high- and the low-distress subject groups, (2) the change in DNA repair over time, and (3) the interaction of these two variables, i.e., the combined comparison of the relative rate and degree of repair for the two groups.

There was a significant difference in DNA repair between the high- and the low-distress subject groups [$F(1,26) = 7.81$, $P < 0.01$], with the high-distress group showing significantly poorer DNA repair, as shown in Fig. 1. There was also the expected significant change over time, which reflected the progressive increase in DNA repair [$F(2,52) = 44.34$, $P < 0.0001$]. The interaction between group membership and change over time approached significance [$F(2,52) = 2.61$, $P < 0.08$].

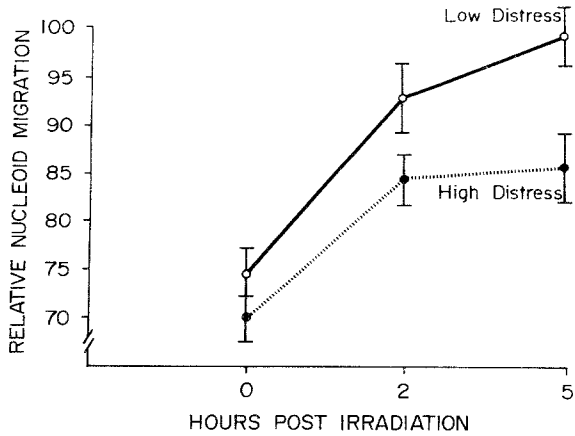

Fig. 1. Mean values for DNA repair (± SE) in lymphocytes from high- and low-distressed psychiatric inpatients at 0, 2, and 5 hr after X-irradiation.

Additional comparisons were made between the extent of DNA repair in this psychiatric inpatient sample and that in 28 sex- and age-matched (± 1 year) controls who had donated blood to the Red Cross. While no self-report distress data were available on these controls, they were not new psychiatric admissions and, therefore, were, as a group, presumably significantly less distressed. In fact, the likelihood that the mean distress level of unselected adults would fall within the upper 1% of the population can be calculated; it is extremely low ($P < 0.001$).

There was a significant interaction in the DNA repair data between change over time and group membership [$F(2,108) = 5.03$, $P < 0.01$], shown in Table IV. Post hoc comparisons (Waller and Duncan, 1969) of this interaction showed nonsignificant differences immediately after irradiation of lymphocytes (time = 0) and 2 hr postirradiation. However, the two groups did differ significantly in the degree of repair when measured postirradiation at the 5-hr end point ($P < 0.01$). While the main effect for group membership did not reach significance [$F(1,54) = 1.68$], there was again a significant increase in DNA repair over time [$F(2,108) = 129.06$, $P < 0.0001$].

Table IV. Relative Nucleoid Migration: Means and Standard Errors for Psychiatric Patients and Blood Donors at 0, 2, and 5 hr After Irradiation

	Hours postirradiation					
	0		2		5	
Psychiatric inpatients	71.24	(1.34)	89.46	(2.40)	92.32	(2.53)
Red Cross blood donors	72.23	(1.81)	90.52	(2.44)	101.01	(1.42)

The preirradiation NSRs were also compared to assess the possibility that the data obtained might result in part from different properties of the nucleoid bodies in high- and low-distressed individuals. The comparison of the high- and low-distressed groups within the psychiatric sample was nonsignificant [$F(1,26) = 1.02$]. Similarly, the psychiatric sample's preirradiation NSRs did not differ significantly from those of the Red Cross blood donors ($F < 1$).

DISCUSSION

The data obtained in this study suggest that a high level of distress is associated with significantly poorer DNA repair in lymphocytes. Lymphocytes from the high-distress psychiatric inpatients had significantly poorer DNA repair in comparison to lymphocytes from low-distress group; the groups did not differ as a function of cancer-related risk variables. When this psychiatric sample was compared with Red Cross blood donor controls, DNA repair in lymphocytes at zero time and 2 hr post-X-irradiation did not differ significantly between the two groups. However, a significant difference was observed after 5 hr of incubation. The 5-hr sample point generally represents the time at which DNA repair would reach at least 100%, i.e., levels comparable to preirradiation values (Stephens and Lipetz, 1985).

Such alterations in DNA repair could have a number of potentially important consequences. Lymphocytes normally respond to antigen or mitogen stimulation with an increase in DNA synthesis and cell replication. If the lymphocyte response to antigen or mitogen stimulation were impaired and therefore less efficient, the ability of the immune response to combat bacterial or viral infections could be compromised. If lymphocytes have a reduced ability to repair those DNA sequences which not only encode particular genes, but also may be regulatory in their expression (Akrigg and Cook, 1980), then it is possible that such highly distressed individuals could be more susceptible to increased infectious desease and cancer, as discussed previously.

NK activity is thought to be important for the destruction of transformed cells. If acute distress or stress can adversely affect NK activity, as recent data suggest (Aarstad *et al.*, 1983; Herberman, 1982; Kiecolt-Glaser *et al.*, 1984a,b; Shavit *et al.*, 1984), then there could be poorer detection and elimination of transformed cells, contributing to the uncontrolled growth of neoplasms. Therefore, distress could potentially contribute to carcinogenesis directly (through faulty DNA repair) as well as indirectly (by affecting immune surveillance or competence) (Fox, 1981).

In this context, it is interesting to note that a significantly higher incidence of cancer has been associated with higher MMPI depression scores in a 17-year prospective study which used over 2000 nonpsychiatric men and

controlled for a number of risk factors (Shekelle *et al.*, 1981). In addition, data suggest that institutionalized psychiatric patients may have a greater incidence of cancer mortality than the general population (Fox, 1978).

A number of researchers have shown that various stressors can have an effect on lymphocyte function at the cellular level. The data obtained in this study suggest that a high level of distress may also be associated with significant and important dysfunctions at the molecular level as well, at least within a psychiatric population. These data provide some preliminary evidence of possible physiological pathways through which psychosocial variables may be directly associated with an increased risk for cancer and infectious disease.

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