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Stress Depresses Interferon Production by Leukocytes Concomitant With a Decrease in Natural Killer Cell Activity

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This study addressed the effects of a commonplace stressful event on interferon production and natural killer (NK) cell activity and numbers. The quantity of interferons (IFN) produced by concanavalin A stimulated leukocytes obtained from 40 medical students during examinations was significantly lower when compared with IFN levels produced by peripheral blood leukocytes (PBLs) taken 6 weeks earlier (baseline). In addition, three different assays measuring NK cells also showed significant decrements during examinations when compared with baseline samples. These assays included (a) lysis of MOLT-4 target cells, (b) percentage of anti-Leu-7$^+$ (NK) cells, and (c) percentage of large granular lymphocytes. Self-report data documented the significantly greater distress associated with examinations in comparison with baseline samples. The data have implications for immunosuppressive disorders and stress-associated illnesses.

Stressful events may have adverse effects on health. The immune system is thought to be an important mediator between aversive events and infectious disease (Ader, 1981; Borysenko, 1984; Coe & Levine, in press).

There is good evidence that natural killer (NK) cell activity has an important role in the body's defense against cancer and viral infections (Herberman, 1982; Herberman et al., 1982). Interferon (IFN) is a major regulator of NK activity, because it can affect both the growth and differentiation of NK cells from their progenitor cells. Moreover, IFN can activate the lytic activity of target-binding cells, enhance cytolysis of target cells, and increase the number of target cells that can be killed by an effector cell. There is also some evidence that NK cells may themselves produce IFN (Herberman, 1982; Herberman et al., 1982).

Earlier studies with rodents suggested that there might be central nervous system mediation of IFN synthesis. Stress-related changes in IFN production were found in virus-infected mice following the application of physical stressors such as shock (Chang & Rasmussen, 1965; Jensen, 1968). Various stressors may also reduce the responsiveness of certain immune functions to IFN stimulation. Pavlidis and Chirigos (1980) found 52% to 75% reductions in macrophage tumoricidal function in interferon-treated mice following restraint. Moreover, data from Spector (1979) suggest that hypothalamic lesions in mice may change IFN responses to antigens. Changes in IFN production could have significant consequences for NK activity and health.

In two recent studies from our laboratory, we found significant decreases in NK cell activity (using the K562 target cell) in medical students during examinations, in comparison with baseline samples obtained one month previously (Kiecolt-Glaser et al., 1984; Kiecolt-Glaser et al., 1986). Self-report data confirmed that significantly greater distress was associated with examinations in comparison with baseline samples.

In this study we measured changes in total IFN production by concanavalin A (Con A) stimulated peripheral blood leukocytes (PBLs). We also measured NK cell lysis and determined the number of NK cells by using two different procedures. We found that the significant increase in distress associated with examinations was related to a depression of interferon production by PBLs, a depression in NK cell lysis, and a decrease in the total number of NK cells.

Method

Subjects

Leukocytes were obtained from students 6 weeks before examinations and again on the first day of final examinations. Forty second-year medical students (mean age = 24.4) volunteered for participation

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in a research project on stress and immunity; all 40 returned for the second sample. The first sample point (baseline) occurred in mid-April after the students' return from spring vacation. The second sample was taken in late May during final examination week. Both samples were taken during the same 1-hour period, thus avoiding possible diurnal fluctuations.

The Brief Symptom Inventory (BSI), administered each time blood was drawn, provided self-report distress data (Derogatis & Spencer, 1982). Students also provided information on any recent health changes, medications, and sleep each time blood was drawn.

Assay for Interferons

Whole blood, treated with ethylenediaminetetraceticacid (EDTA) to prevent clotting, was placed on Hypaque-Ficoll gradients to separate the mononuclear cells. Individual PBL samples of 5×10^6 mononuclear cells were suspended in complete RPMI 1640 medium supplemented with 0.25 mg/ml of human serum albumin. Induction of IFNs in each culture was accomplished by the addition of 10 μg of Con A (Calbiochem) and incubation at 37 °C for 48 hours. Supernatants from individual cultures were assayed by a viral cytopathic effect (CPE) reduction assay, which used vesicular stomatitis virus (VSV) and HEP-2 cells (Stewart, 1979). Each well was challenged with 1,000 plaque-forming units of VSV (Indiana strain). Interferon titers were defined as the reciprocal of the highest dilution that provided 50% protection against VSV-induced CPE. By using human cells we are detecting primarily IFN-γ, but we cannot rule out detecting some IFN-α as well (Wiranoska-Stewart, 1981). Commercially prepared IFN-γ (Meloy) was included as a standard in each assay and gave 50% protection at 3 U/ml. National Institutes of Health reference standard IFN-α gave 50% protection at 3 U/ml.

Quantitation of NK Cells and NK Cell Lysis

The percentage of NK cells and NK cell lysis in each blood sample was determined. Adherent cells were removed from the PBL preparation by placing the cell suspensions in plastic tissue culture flasks and incubating at 37 °C in a CO_2 incubator for 1–3 hours. The nonadherent cells were washed off and used to determine percentage of anti-Leu-7^+ NK cells. Lymphocytes (10^6) were incubated in .01 ml of anti-Leu-7 (HNK-1) monoclonal antibody for 30 min on ice (Beckton-Dickinson Monoclonal Center, 1982). Cells were washed and resuspended in goat anti-mouse IgG conjugated to fluorescein isothiocyanate (Cappel Laboratories) and incubated for an additional 30 min on ice. The cells were washed and assayed using an Ortho System 50 fluorescence activated cell sorter (FACS).

The percentages of large granular lymphocytes (LGLs) were measured by a 100 cell differential count on air-dried slides prepared with Wright Giemsa staining. Cells counted as LGLs were large lymphocytes with pale and characteristic granular cytoplasm (Timonen, Saksela, Ranki, & Hayry, 1979). The percent NK cell lysis was determined using MOLT-4 cells as targets by procedures described previously (Kiecolt-Glaser et al., 1984; Zaretskaya, Burkhanov, Dolbin, & Metodiev, 1983).

Results

Self-Report Data

The immunological and self-report data were analyzed using a repeated measures analysis of variance (ANOVA). There was one within-subjects independent variable, change from the baseline sample to the examination sample.

The General Severity Index of the BSI increased from a t-score mean of 56.75 to 61.17, $F(1, 39) = 12.25$, $p < .001$, which documented significantly increased distress associated with examinations in comparison with baseline samples. Students reported a slightly larger sleep deficit in the 72 hr preceding the examination blood draw (3.47 hours) than in the comparable period before baseline (0.11 hr), $F(1, 39) = 15.78$, $p < .001$. The size of the sleep deficit was not significantly correlated with any of the immunological measures, with correlations ranging from −.24 to .01 during examinations.

Changes in Interferon Production, NK Cell Numbers, and NK Cell Lysis

Production of IFNs by Con A stimulated lymphocytes declined sharply from the first to the second sample, $F(1, 39) = 106.13$, $p < .0001$, as shown in Table 1. We also measured IFN levels in plasma from both blood samples. No subject had measurable levels of plasma IFNs at either sample point.

As shown in Table 2, the percentage of anti-Leu-7^+ (NK) cells declined significantly in PBL samples obtained during examinations, as compared with the PBL samples obtained at baseline, $F(1, 39) = 41.73$, $p < .0001$. Similar results were obtained in the percentage of LGLs, $F(1, 39) = 6.58$, $p < .01$. The NK cell lysis of MOLT-4 cells was analyzed using a two-factor repeated measures ANOVA, with two within-subjects independent variables: change from baseline to examinations and changes across the three effector to target cell ratios. NK cell lysis decreased significantly from the first to the second blood sample, $F(1, 39) = 8.14$, $p < .01$, as shown in Figure 1. There were also the expected significant differences across the three ratios, $F(2, 76) = 14.07$, $p < .0001$. The interaction between these two variables was not significant, $F < 1$, which indicates that the size of the decrement was fairly constant across the three different cell ratios.

Nutritional Status

In order to assess the possibility that the decrease in the synthesis of IFNs by PBLs and the depression of NK cell numbers and lysis were due to poor nutrition prior to the second blood sample, we measured plasma levels of albumin, transferrin, and retinol-binding protein in both samples (Doumas, Watson, & Biggs, 1971; Keyser, 1979; Rodkey, 1965); the association between poor nutrition and cellular immunity

Table 1
Means (± SE) of IFNs Produced by PBLs Stimulated With Con A and Plasma IFN Levels at Baseline and During Examinations

Sample	Leukocyte IFNs U/ml	Plasma IFNs U/ml
Baseline		
M	2,003.03	0
SE	179.13	0
Examinations		
M	80.00	0
SE	17.99	0

Note. IFN = interferon. PBL = peripheral blood leukocytes.

Table 2
Means (± SE) for Percentages of Anti-Leu-7⁺ Cells and LGLs in Blood Samples Taken at Baseline and During Examinations

Sample	Percent Anti-Leu-7⁺ Cells	Percent LGLs
Baseline		
M	16.33	3.95
SE	1.31	0.30
Examinations		
M	9.09	3.17
SE	2.20	0.24

Note. LGL = large granular lymphocytes.

is well documented (Chandra & Newberne, 1977). The nutritional assay means at baseline and during examinations were 4.56 g/dl and 4.87 g/dl for albumin, 320.61 mg/dl and 387.03 mg/dl for transferrin, and 3.34 mg/100 ml and 5.73 mg/100 ml for retinol-binding protein. All three markers were within the normal range at both sample points, which strongly suggests that the depressions in IFN production, NK cell numbers, and NK cell lysis were not a function of poor nutrition.

Discussion

The data obtained in this study demonstrate a very large and significant decrease in the amount of IFNs produced by Con A stimulated PBLs obtained from medical students during examinations, in contrast to the baseline values obtained 6 weeks earlier. There was also a significant decrement in the activity of NK cells, as measured by lysis of MOLT-4 target cells. A different NK target cell (MOLT-4) was used in this study than we had used in our earlier reports (K-562), in order to assess the generality of stress-related changes across target cells and to rule out the possibility that we were originally measuring unique changes in a subpopulation of NK cells (Kiecolt-Glaser et al., 1984; Kiecolt-Glaser, Glaser, et al., 1985; Kiecolt-Glaser et al., 1986).

The two assays used to quantify the number of NK cells (percent Leu7⁺ cells and percent LGLs) showed significant declines during examinations. Similar results were obtained

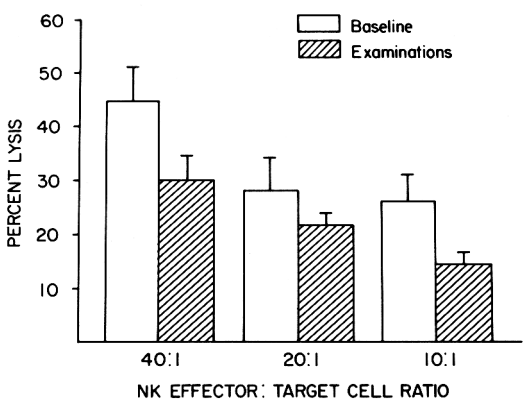

Figure 1. Means (± *SE*) for percent lysis of MOLT-4 cells for the three natural killer (NK) effector to target cell ratios at baseline and during examinations.

when the absolute numbers of Leu7⁺ NK cells for each person and for each effector to target cell ratio was calculated. These data suggest that the decrease in NK cell lysis reported earlier in animal and human studies may be due, at least in part, to a decrease in total number of NK cells. The reproducibility of these decrements in human NK activity across human subject samples and target cells, together with the similar data obtained in animal studies following physical stressors (Aarstad, Gaundernack, & Seljelid, 1983; Shavit, Lewis, Terman, Gale, & Liebeskind, 1984), provides good evidence for psychosocial modulation of NK cell activity.

Additional evidence for the reliability and pervasiveness of the immunodepressive effects of acute stress is seen in data from our earlier studies with medical student subjects. For example, we found significantly higher antibody titers to three latent herpesviruses during final examinations, in comparison with lower titers after the students' return from summer vacation; moreover, the lonelier students had higher antibody titers to Epstein-Barr virus (Glaser, Kiecolt-Glaser, Speicher, & Holliday, 1985). Higher antibody titers to latent herpesviruses suggest poorer cellular immune system control over virus latency. We have also found decreased responsiveness of T-lymphocytes to mitogen stimulation in medical student blood samples obtained during examinations, as well as lower percentages of total T-lymphocytes. Similarly, blood samples obtained during examinations showed lower percentages of two T-cell subpopulations, helper and suppressor cells, than blood samples obtained at baseline (Glaser, Kiecolt-Glaser, Stout, et al., 1985).

There is no evidence that the immunological changes found in these medical student studies were simply artifacts of the minor variations in nutrition or sleep. In addition, the absence of measurable levels of plasma IFNs in this study and the students' reports of good health suggest that the immunological changes were not a function of other health problems such as virus infections in this otherwise healthy subject sample.

These IFN and NK data may have important health implications. The modified theory of immune surveillance suggests that cancer cells can develop spontaneously in the body but are normally destroyed by the immune system; NK cells are thought to be an important host defense in this regard (Herberman, 1982; Herberman et al., 1982). Our data suggest that this host defense can be significantly modified by a relatively commonplace stressor.

In this context, the repair of DNA damaged by carcinogens is an important process, because poorer DNA repair is associated with an increased incidence of cancer (Setlow, 1978). We have previously found a defect in DNA repair in lymphocytes from very depressed psychiatric inpatients (Kiecolt-Glaser, Stephens, Lipetz, Speicher, & Glaser, 1985), and we have also shown that rotational stress can alter production of an important DNA repair enzyme (Glaser, Thorn, Tarr, Kiecolt-Glaser, & D'Ambrosio, 1985). It appears that stress could contribute directly to carcinogenesis through impairments in the DNA repair process, as well as indirectly by poorer destruction of mutant or transformed cells.

It is interesting to note that highly distressed populations such as bereaved spouses and psychiatric patients also have a

greater incidence of cancer mortality than the general population (Bloom, Asher, White, 1978; Ernster, Sacks, Selvin, & Petrakis, 1979; Fox, 1978). Morever, in a 17-year prospective study using over 2,000 nonpsychiatric men, higher levels of depression were associated with a significantly higher incidence of cancer, even after correction for a number of relevant risk factors (Shekelle et al., 1981). Therefore, longer-term stress-related alterations in cellular immunity may carry an increased risk for immunodeficiency disorders and malignant and infectious disease.

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