Stress-Related Impairments in Cellular Immunity

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Abstract. The percentages of total T-lymphocytes (OKT-3+), helper T-cells (OKT-4+), and suppressor T-cells (OKT-8+) were significantly lower in blood samples obtained from 40 medical students during examinations, compared to baseline values obtained 6 weeks earlier. In addition, the response of T-lymphocytes to stimulation by phytohemagglutinin and concanavalin A was also significantly lower during examinations, compared to baseline. Self-report data documented significantly greater distress associated with examinations. The data have implications for immunosuppressive disorders and stress-related illnesses.

Key Words. Stress-related impairment, helper cells, suppressor cells, blastogenesis.

There is growing evidence suggesting that stressful events may significantly affect the body's resistance to infectious and malignant disease. Recent research suggests that such effects are mediated through the immune system (Ader, 1981). For example, there are well-documented stress-related decreases in the T-lymphocyte response to mitogen stimulation following such stressful events as bereavement (Bartrop et al., 1977) and astronauts' space flights (Kimzey, 1975). Clinical symptoms may accompany these stress-related immunological changes (Cohen-Cole et al., 1981).

Work from our laboratory has addressed the effects of relatively commonplace stressful events on the immune response. We found significantly lower levels of natural killer (NK) cell activity in blood samples obtained from medical students during final examinations, compared to baseline samples taken 1 month earlier (Kiecolt-Glaser et al., 1984a). Antibody levels to latent Epstein-Barr virus (EBV), Herpes simplex virus (HSV), and cytomegalovirus (CMV) were also significantly affected by this academic stressor, presumably as a function of underlying changes in cellular immunity (Glaser et al., in press).

In this study we wished to determine if there were stress-related changes in the percentages of total T-lymphocytes, helper, and suppressor cells; such changes could have implications for risk for disease and health in general. Helper cells have an...
inducer function for the B-lymphocyte proliferation and differentiation sequence that is critical for the synthesis of immunoglobulins. The optimal development of toxicity in T5+ antigen effector cells (cytotoxic/suppressor cells) requires the presence of helper cells. Helper cells also play an inducer role in the interactions between T-lymphocytes and macrophages. Therefore, if helper cell functions are disrupted, immunodeficiency may result. In contrast, autoimmune disorders are associated with alterations in suppressor cell functioning (Reinherz and Schlossman, 1980). Changes in the relative percentages of helper and suppressor cells are thought to be important in screening for disease, and in determining the relative efficacy of various experimental treatments for immunodeficient disorders such as the acquired immune deficiency syndrome (AIDS) (Pitchenik et al., 1983).

Methods

Subjects. Forty volunteers (15 females and 25 males) were recruited from the second-year medical student class for a research project on stress and immunity. Blood samples were drawn twice from all subjects, with the first draw occurring 6 weeks before final examinations (baseline), and the second during final examinations. The baseline blood draw was scheduled during the first week after the students returned from their spring vacation. The blood draws were scheduled for the same 1-hour period each time, avoiding possible diurnal fluctuations.

Self-report data were collected each time blood was drawn. The Brief Symptom Inventory (BSI) (Derogatis and Spencer, 1982), a reliable and valid self-report measure of distress, provided information on changes in stress-related symptoms from the first to the second sample point. Students also provided information on recent health changes, medications, weight, and sleep each time blood was drawn.

T-Lymphocyte Subset Assay. The percentages of helper/inducer T-cells, suppressor/cytotoxic T-cells, and helper/suppressor cell ratios were determined using the monoclonal antibodies OKT-4 and OKT-8, respectively (Ortho), as previously described (Moll et al., 1982). The OKT-3 monoclonal antibody was also used to measure the total number of circulating OKT-3+ T-lymphocytes.

Briefly, lymphocytes isolated on Hypaque-Ficoll gradients were washed with trypsin diluent, then resuspended in complete RPMI 1640 medium supplemented with 20% fetal bovine serum. Monocytes were removed by placing the cell suspensions in plastic tissue culture flasks and incubating at 37°C in a CO₂ incubator for 2 hours. The nonadherent cells were washed off and used to determine percentage of T-cell subsets. Lymphocytes (10⁶) were incubated in 0.01 ml of OKT-3, OKT-4, or OKT-8 monoclonal antibody for 30 minutes on ice. Cells were washed with cold RPMI 1640/PBS (1:1), resuspended in goat anti-mouse IgG conjugated to fluorescein isothiocyanate (Cappel Laboratories), and incubated for an additional 30 minutes on ice. The cells were washed and assayed, using an Ortho System 50 fluorescence activated cell sorter (FACS).

Blastogenesis. Mitogens were used at a final concentration of 2.5, 5.0, and 10.0 µg/ml for concanavilin (Con A) and 0.25, 0.5, 1.0, and 2.0 µg/ml for phytohemagglutinin (PHA). Each assay was performed in triplicate. Complete medium supplemented with 20% fetal bovine serum was used for baseline controls. One-tenth ml of mitogen was added to 1 x 10⁵ lymphocytes (in 0.1 ml complete medium) in 96 well plates, and incubated at 37°C for 48 hours. Fifty microliters of tritiated thymidine (10 µCi/ml, specific activity 83 Ci/mM) were added to each well and the plates incubated at 37°C for 4 hours. Cells were harvested onto GF/A filters. Radioactivity was measured using a Beckman LS7000 scintillation counter. The data are presented as the counts per minute (c.p.m.) in the stimulated samples minus the c.p.m. of the unstimulated samples (Δ c.p.m.). A logarithmic transformation was performed on the resulting values.
Results

Initial repeated measures analysis of variance (ANOVA) tests revealed no differential sex of subject effects for the major dependent immunological and self-report variables, so data from males and females were combined for the main analysis. Therefore, unless otherwise indicated, all self-report and immunological data were analyzed using a repeated measures ANOVA, with one within-subjects independent variable, change from the baseline to the examination sample.

Self-Report and Nutritional Data. Self-reported distress increased significantly from the first to the second sample, as shown in the T-score data presented in Table 1. There were significant changes on two global distress indices (the General Severity Index and the Positive Symptom Total), as well as in most of the symptom scales. These data document the increased distress associated with examinations.

The students also reported minor changes in sleep, with a mean of 23.89 (SD = 2.37) hours in the 3-day period before the baseline blood drawing, and a mean of 20.34 (SD = 3.95) in the 3-day period before the examination blood drawing ($F = 23.81; df = 1, 39; p < 0.001$). However, sleep was not significantly correlated with the immunological measures at either sample point.

While students described common stress-related symptoms (e.g., headaches and stomach aches), no student reported any fever or other symptoms associated with infectious disease during the 2-week period preceding baseline and the comparable time period before examination. Similarly, students’ responses to questions about medications did not include any which might have had obvious immediate or enduring consequences for immune function, i.e., most students reported no medication usage, and the minority who did report taking medication primarily listed vitamins and aspirin.

Table 1. Brief Symptom Inventory (BSI) distress T-scores at baseline and during examinations

<table>
<thead>
<tr>
<th>BSI Scale</th>
<th>Baseline</th>
<th>Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Somatization</td>
<td>49.36</td>
<td>8.37</td>
</tr>
<tr>
<td>Obsessive-compulsive symptomatology¹</td>
<td>57.47</td>
<td>10.09</td>
</tr>
<tr>
<td>Interpersonal sensitivity</td>
<td>56.11</td>
<td>8.89</td>
</tr>
<tr>
<td>Depression²</td>
<td>55.97</td>
<td>9.08</td>
</tr>
<tr>
<td>Anxiety³</td>
<td>55.47</td>
<td>9.08</td>
</tr>
<tr>
<td>Phobic anxiety</td>
<td>55.47</td>
<td>8.70</td>
</tr>
<tr>
<td>Hostility²</td>
<td>53.39</td>
<td>12.38</td>
</tr>
<tr>
<td>Paranoia</td>
<td>50.64</td>
<td>8.48</td>
</tr>
<tr>
<td>Psychoticism</td>
<td>57.36</td>
<td>10.41</td>
</tr>
<tr>
<td>General Severity Index¹</td>
<td>56.75</td>
<td>9.35</td>
</tr>
<tr>
<td>Positive Symptom Total³</td>
<td>53.36</td>
<td>8.40</td>
</tr>
<tr>
<td>Positive Symptom Distress Index</td>
<td>56.31</td>
<td>9.02</td>
</tr>
</tbody>
</table>

¹. $p < 0.001$.
². $p < 0.01$.
³. $p < 0.0001$. 
Since it is known that nutrition can affect cellular immunity (Chandra, 1981), we performed assays to measure plasma levels of albumin, transferrin, and retinol-binding protein (Howard and Meguid, 1981). We found that all three nutritional markers were within normal limits at both sample points (data not shown).

**T-Lymphocyte Subsets and Blastogenesis.** There were significant decreases in the percentages of OKT-3+ T-lymphocytes \((F = 8.97; df = 1, 39; p < 0.01)\), helper cells \((F = 4.17; df = 1, 39; p < 0.05)\), and suppressor cells \((F = 5.90; df = 1, 39; p < 0.05)\). However, the helper/suppressor ratio did not change significantly. The percentage of total lymphocytes was also determined by Coulter Counter using standard clinical laboratory procedures. The mean percentage of lymphocytes decreased significantly \((F = 10.86; df = 1, 39; p < 0.01)\). These data are shown in Table 2.

### Table 2. Percentages of total lymphocytes, OKT-3+ T-lymphocytes, helper cells, suppressor cells, and the helper/suppressor ratio

<table>
<thead>
<tr>
<th>Blood samples</th>
<th>% Lymphocytes</th>
<th>% OKT-3+ T-lymphocytes</th>
<th>% Helper cells</th>
<th>% Suppressor cells</th>
<th>Helper/suppressor ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Baseline</td>
<td>34.53</td>
<td>7.09</td>
<td>57.20</td>
<td>9.34</td>
<td>40.41</td>
</tr>
<tr>
<td>During exams</td>
<td>30.03</td>
<td>7.50</td>
<td>47.59</td>
<td>12.62</td>
<td>36.37</td>
</tr>
</tbody>
</table>

1. \(p < 0.01\).
2. \(p < 0.05\).
3. Nonsignificant.

The normal range for OKT-3+ cells is 61-79%, for OKT-4+ (helper/inducer) cells is approximately 34-54%, for OKT-8+ (suppressor/cytotoxic) cells is 20-37%, and for OKT-4/OKT-8 cell ratios is 1.1-3.5. The normal mean for the percentage of lymphocytes in normal adults in our clinical laboratory is 34.

The T-lymphocyte response to stimulation by Con A and PHA was also significantly lower during examinations. Two within-subjects variables were included in the repeated measures ANOVA used to analyze the mitogen data, change over samples and mitogen concentration. There was a significant decrease in response to Con A in the examination sample across mitogen concentrations \((F = 106.68; df = 1, 39; p < 0.0001)\), as shown in Fig. 1a. There were also significant increases in lymphocyte responsiveness associated with the increasing Con A concentrations \((F = 467.59; df = 2, 78; p < 0.0001)\), as well as a significant interaction between Con A concentration and change from baseline to examinations \((F = 14.31, df = 2, 39; p < 0.0001)\). The interaction reflects the greater differences found between the baseline and examination mitogen responsiveness at lower Con A concentrations.

We also found a significant decrease in the lymphocyte response to PHA during examinations when compared to baseline \((F = 5.51; df = 1, 39; p < 0.03)\) (Fig. 1b). Greater T-lymphocyte responsiveness was associated with higher mitogen concentrations \((F = 191.13; df = 1, 39; p < 0.0001)\), while the interaction between concentration and change from the first to the second sample was not significant \((F = 1.46; df = 3, 117; NS)\).
Fig. 1. Mean (± SEM) T-lymphocyte response to mitogen stimulation at baseline and during examinations

![Graph showing response to Con A and PHA](image)

**Discussion**

In this study, we examined the effect of a stressful event on certain aspects of cellular immunity. We found significant decreases in the percentages of lymphocytes, total T-lymphocytes (OKT-3+), helper cells, and suppressor cells. We also found a significant decrease in T-lymphocyte proliferation in response to stimulation by PHA and Con A. Self-report data documented the significantly increased distress associated with examinations.

It is of interest that the mean percentage of OKT-3+ lymphocytes at baseline (57.20) is below the normal range obtained with the OKT-3+ monoclonal reagent, which is between 61% and 79%. It is possible that the students had some immune deficiency at baseline, since they had already gone through most of the academic year and had been exposed to a number of high- and low-level stressful events. Data obtained in a previous study examining the effect of examination stress on latent herpes viruses support this interpretation (Glaser et al., in press).

Overall, these immunological data document significant changes associated with a stressful event (examinations), in comparison to the baseline levels measured 6 weeks previously. There is no evidence that these changes were artifacts of the relatively minor variations in nutrition or sleep. In addition, the students’ reports of good health suggest that the immunological changes were not a function of other health problems in this subject sample.

Consistent with the data from this study, data from other laboratories also suggest that lowered responsiveness to mitogen stimulation is associated with depressive illness (Schliefer et al., 1984), as are lower percentages of lymphocytes (Kronfol et al., 1984). The consistency of such findings across diverse subject groups suggests that greater distress is associated with poorer immune function.

Our data contrast with those of Baker et al. (1984), who suggested that an increased percentage of OKT-4 (helper/inducer) T-lymphocytes was part of a physiological reaction to stress. However, they based that conclusion on data from two different medical student classes which were compared at a single point in time, rather than on longitudinal data collected over high- and low-stress periods.
Consistent with the data from our previous studies of medical students (Kiecolt-Glaser et al., 1984a, 1984c; Glaser et al., in press) and psychiatric inpatients (Kiecolt-Glaser et al., 1984b; Kiecolt-Glaser et al., in press), these new data further document the significant impact of a commonplace stressor on a number of different facets of the immune response. Particularly striking is the consistency of these effects across different medical student classes (Kiecolt-Glaser et al., 1984a). Despite these students’ long histories of successful examination performance, there are still reliable and significant changes in a number of different cellular immune system parameters, even at the end of the second year of medical school. While these immunological changes were not immediately associated with an increase in illness in this young and healthy sample, such immunological changes might have important consequences in individuals whose health is already impaired, in individuals who are exposed to an infectious agent or carcinogen, in individuals who already have latent viruses or undetected tumor cells, or in older populations which have age-related decrements in immune competence. In these and other at-risk groups, novel and intense stressful events may affect morbidity and mortality.

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References