Published in final edited form as:

Brain Behav Immun. 2005 May ; 19(3): 243–251.

Preliminary evidence for lymphocyte distribution differences at rest and after acute psychological stress in PTSD-symptomatic women[☆]

Dorie A. Glover^{a, *}, Amber C. Steele^a, and Margaret L. Stuber^a

*a*Division of Child and Adolescent Psychiatry, Department of Psychiatry and Biobehavioral Sciences, UCLA Neuropsychiatric Institute, Los Angeles, CA 90024-7159, USA

John L. Fahey^b

*b*Microbiology, Immunology and Molecular Genetics-CIRID/Med-CIA, UCLA Geffen School of Medicine, Los Angeles, CA, USA

Abstract

This study investigated circulating natural killer (NK), CD4+ and CD8+ cells in response to acute psychological challenge among mothers of child cancer survivors with and without posttraumatic stress symptoms (PTSS). Control mothers of healthy children ($n = 9$) were compared to 17 cancer mothers with (PTSS: $n = 9$) and without PTSS (No PTSS: $n = 7$) under conditions of rest, after a generic stressor (MAT: mental arithmetic task) and a personalized stressor (script-driven trauma imagery), and after recovery from each stressor. Results indicate the PTSS group had higher percentage CD4+ and lower CD8+ levels than non-symptomatic women and blunted NK reactivity to generic challenge. Multiple regression analyses indicated PTSS effects were independent of self-reported distress. Contrary to expectations, cancer mothers without PTSS were not significantly different from controls on tonic or phasic immune outcomes. Also unlike predictions, reactivity to challenge was greatest to the non-social MAT stressor compared to the personalized challenge for all groups. Conclusions are constrained by study limitations (e.g., small sample size and potential phase order effects). Nonetheless, results are consistent with an emerging literature on PTSS-associated immune differences and further suggest these effects may be distinct from that associated with subjective distress more generally.

Keywords

Posttraumatic stress disorder; Stress; Women; Laboratory challenge; Natural killer

1. Introduction

Although it is widely recognized that exposure to “stress” alters lymphocyte distribution (Herbert and Cohen, 1993; Kiecolt-Glaser et al., 2002) surprisingly few studies have reported immune data from individuals with posttraumatic stress disorder (PTSD). Four controlled studies could be found: [Male combat veterans: Boscarino and Chang, 1999; Laudenslager et al., 1998; Adult female child abuse victims: Wilson et al., 1999; Mixed gender natural disaster victims: Ironson et al., 1997]. Only CD4+ and CD8+ cells were reported in all studies. Two

[☆] This research was made possible by support from: National Institute of Mental Health (#1K01-MH01939-01A2), American Cancer Society (#PF-4480), Norman Cousins program of Psychoneuroimmunology at UCLA (#34323), and General Clinical Research Center, UCLA Geffen School of Medicine (#5M01RR00865-25).

*Corresponding author. Fax: +310 206 3373. E-mail address: dglover@mednet.ucla.edu (D.A. Glover).

studies (Laudenslager et al., 1998; Wilson et al., 1999) found no PTSD-related group differences, one study found PTSD-related reductions (Ironson et al., 1997), while another reported elevations (Boscarino and Chang, 1999) in CD4+ and CD8+ counts under resting conditions.

The lack of consistency in results across these studies may be due to differences in gender, stressor type, and timing of the assessment in relation to trauma onset. In addition, examination of lymphocyte redistribution following laboratory challenge suggests that sometimes group differences are evident only when subjects are acutely stressed. For example, blunted natural killer (NK) cell cytotoxicity and B-endorphin response, increased epinephrine and NK cell number and slow cortisol recovery were found in response to an acute psychological stressor in men reporting a high degree of “life stressors” as compared to men reporting a low degree of “life stressors” (Pike et al., 1997). In a study of male adults reporting very high or very low daily “hassles”, blunted NK cell number among the high stress group was reported in response to a brief psychological stressor (Benschop et al., 1994). Likewise, college students who scored high on a measure of trait “worry” had a blunted NK response to fear provocation compared to low worriers (Segerstrom et al., 1999).

These laboratory challenge studies found no group effects under resting conditions, but immune differences emerged across low and high stress groups under challenge. Extending a challenge procedure to individuals with PTSD symptoms may help to clarify the inconsistent immune findings of previous resting condition studies. Furthermore, the use of a challenge procedure among those with a history of high life stress with and without current PTSD symptoms may help determine whether lymphocyte distribution in PTSD is distinct from the pattern for high life stress alone.

Mothers of healthy children (controls) were compared to mothers of child cancer survivors with and without posttraumatic stress symptoms (PTSS). All cancer mothers can be characterized as having a high stress history due to frequent acute and prolonged stress associated with their child’s cancer diagnosis and treatment (Stuber et al., 1998). However, only a subset of cancer mothers show clinically significant posttraumatic stress symptoms (PTSS) (Stuber et al., 1996). Based on previous immune studies, we first predicted baseline (resting) group differences in lymphocyte distribution across PTSS versus non-symptomatic women. Due to the equivocal nature of CD4+ and CD8+ resting levels in previous studies of PTSD, we did not predict a direction for these resting group differences. Second, based on the life stress challenge studies, we hypothesized that all cancer mothers would show blunted immune responsivity (i.e., smaller change in NK, CD4+ and CD8+ cells following challenge) relative to control mothers. Finally, because interpersonal stressors appear to have a greater impact on lymphocyte distribution than non-social stressors (Herbert and Cohen, 1993), lymphocyte distribution was tested following a personalized challenge (individualized trauma imagery) and after a generic challenge (mental arithmetic). This allowed us to determine if group differences were limited to interpersonal (high impact) stressors or would emerge even under generic stress.

2. Methods

2.1. Subjects

Mothers of child cancer survivors were identified through tumor registry information collected on their children at the University of California, Los Angeles (UCLA) Medical Center. Cancer mothers were asked to participate only if their child was (a) alive; (b) off active cancer treatment; (c) considered a cancer “survivor” with no current relapse. Of 80 respondents to a recruitment letter that met eligibility criterion, 66 (82.5%) participated in a telephone interview (Phase I), which assessed for PTSD symptoms and general health. All subjects were asked to

participate in Phase II (laboratory challenge) during the telephone interview. Some subjects were no longer in the geographic area and others met Phase II exclusion criteria: illness or medication histories that might influence immune parameters or self-reported substance use (tobacco, alcohol or drugs). The final sample of laboratory challenge participants consisted of 17 cancer mothers. Control mothers of healthy children ($n = 9$) were recruited via advertisements at the UCLA Medical Center. All subjects were paid \$80 for participation, which included the laboratory challenge and (on separate days) the collection of a 12-h overnight urine sample for neuroendocrine analysis. Further details of subject recruitment and characteristics have been reported elsewhere (Glover and Poland, 2002; Glover et al., 2003). The study was approved by the UCLA Institutional Review Board.

2.2. Assessing PTSD and depression

All subjects were assessed with the Posttraumatic Stress Diagnostic Scale (PDS) (Foa, 1995; Foa et al., 1997) and Beck Depression Inventory (BDI) (Beck and Steer, 1984). Neither are clinical interviews, but both are widely used research instruments consistent with the diagnostic criteria of DSM-IV (American Psychiatric Association, 1994). The BDI yields a symptom severity score. Scores above 13 are generally considered clinically significant. The PDS yields a categorical diagnosis (meets all DSM-IV criteria for PTSD) and a symptom severity score useful for assessing subthreshold symptoms.

2.3. Assessing procedural distress

Upon arrival and throughout the laboratory session (see below), subjects were asked to report how distressed they felt at that moment on a subjective units of distress scale (SUD), ranging from 0 (not at all distressed) to 8 (extremely distressed).

2.4. Procedures

Subjects were instructed to refrain from medication, coffee or substance use for 24 h prior to the laboratory session and were asked to refrain from eating for 2 h prior to the laboratory session. They were also screened for these behaviors upon arrival. All laboratory sessions occurred in the early evening hours (1700). First, subjects reported current distress (SUD rating), then height, weight, blood pressure, and pulse were measured. Spot electrodes were attached to assess heart rate and vagal tone and a 20-gauge angiocatheter (IV) was inserted into antecubital vein. After at least 1 h of habituation to the environment and a SUD rating, subjects underwent a 5-min baseline recording period, a generic challenge, a recovery period, an individualized trauma challenge, and a final recovery period. Each phase lasted approximately 12-15 min. Subjects gave a SUD rating immediately after each phase.

In the generic mental arithmetic test (MAT), subjects were asked to count backwards by 7's, beginning with 4500. A metronome was set at a fast pace, to increase psychological pressure for subjects to calculate quickly. In addition, a research assistant stood in front of the seated subject, giving feedback on accuracy and prompting subjects in a stern tone to "go faster please." The individualized trauma imagery challenge followed procedures from Shalev et al. (1993). Personalized scripts were composed to help subjects invoke memories of their trauma experience. To equate the trauma imagery for control mothers whose children had no serious illness, controls were asked to select and describe a traumatic event. All control subjects reported a serious trauma (e.g., witnessing the death of a family member, automobile accident). Using the scripts, subjects were guided through an imagery session of their "worst moments." During the final recovery period subjects were left alone and instructed to select an activity that would help them "return to normal." They were provided with a video, reading material, music, and a game.

2.5. Immune measures

Cell percentages in the following lymphocyte subsets were assessed via enumerative assays: CD4+ (helper T), CD8+ (cytotoxic T) and CD56+16+ (natural killer). Whole blood was maintained at room temperature until flow cytometry. Fifty microliters of well-mixed EDTA whole blood was incubated with 10 μ l of fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- and PerCP-conjugated murine antihuman monoclonal antibodies (Beckton-Dickinson, San Jose, CA) for 15 min at room temperature (22-25° C). After incubation, red blood cells were lysed with FACS Lyzing solution (Beckton-Dickinson) and incubated for 10 min in the dark at room temperature, after incubation the tubes centrifuged, and the supernatant aspirated without disturbing cell pellet. Finally, the cell pellet washed by adding 1.6 ml of wash buffer, and immediately the stained samples were analyzed with a FACScan flow cytometer (Beckton-Dickinson, San Jose, CA) equipped with a 15-W argon laser. Isotype controls were used to evaluate nonspecific binding and to position cursors. Lymphocytes were identified by gating on low forward scatter (related to cell surface area) and 90° side scatter (related to cell granularity). Data collection and data analysis are done with CELLQuest Software (Beckton-Dickinson).

2.6. Data analysis

Data were analyzed by a 3 (Group: Cancer with and without PTSS, Controls) $\times$ 5 (Phase: Baseline, MAT Stressor, Recovery 1, Imagery Stressor, Final Recovery) repeated-measures analysis of variance (SPSS for Windows 10.1). Significant group effects were examined with planned contrasts comparing each cancer group to controls and a post hoc contrast comparing cancer groups to each other, overall and at each phase (individual phase effects referred to as results of parameter estimates). Phase effects were analyzed as polynomial contrasts at Order 4, representing the expected patterns over time. Significant group $\times$ phase interactions were followed by examination of change scores to control for baseline group differences.

Potential health modifiers that correlated with an immune indicator (Pearson's $r > .30$) were included as statistical covariates in all analyses of that indicator. The following variables were examined: age, and for the last 24 h, number of alcohol and caffeinated drinks (e.g., coffee) consumed, minutes of aerobic exercise and a rating of whether the amount of sleep the previous night was adequate (yes or no). F and p values for all variables in each analysis are provided.

CD8+ cells were generally reciprocal of CD4+ cells across number and percentage, thus we report only CD4+:CD8+ ratio statistical analyses. ANCOVA assumptions of normality and homogeneity of variance were met for NK and total lymphocytes, but CD4+:CD8+ ratios for each time period were transformed with a reciprocal transformation before analysis to correct for violations in homogeneity of variance.

3. Results

3.1. Subjects

The PDS assessment showed all cancer and control mothers reported having lived through or witnessed at least one traumatic event in the past and most reported multiple traumas. All subjects were asked to select the event that “bothers you the most” and answer all subsequent questions about PTSD symptoms with respect to that “worst” event. All cancer mothers reported their child's cancer as the worst trauma experienced. Of 17 cancer mothers, 8 met all DSM-IV criterion (A-F) for PTSD and 2 met subthreshold criteria (developed by Hawk et al., 2000). Remaining cancer mothers ($n = 7$) and controls ($n = 9$) did not meet categorical criteria for PTSD. One of the 8 cancer mothers meeting all criteria had few symptoms, low symptom severity, and mild functional impairment on the PDS. All other subjects showed consistency across these dimensions (i.e., clearly no/low or moderate/severe PTSS). This subject was

dropped from the analyses due to the questionable diagnostic status. The final laboratory sample ($n = 25$) consisted of 3 groups: (1) *PTSS*: Cancer mothers with moderate to severe posttraumatic stress symptoms ($n = 9$); (2) *No PTSS*: Cancer mothers with no/low symptoms ($n = 7$); and (3) *Control*: Mothers of healthy children ($n = 9$). The rate of PTSD among Phase II laboratory challenge cancer mothers (7/16 or 44%) was similar to the larger Phase I telephone sample (27/66 or 41%). BDI depression scores were clinically significant (above 13) in 55.6% of PTSS mothers (5 of 9), but not in any No PTSS or control mothers.

As expected, groups were significantly different on the number of PTSD symptoms and symptom severity on the PDS. See Table 1. PTSS mothers had more symptoms [$F(2,24) = 31.91, p < .001$] and greater symptom severity [$F(2,24) = 19.44, p < .001$] than the other two groups, who did not differ. The number of years since the “trauma” (for cancer mothers, this was taken as the time since their child’s cancer diagnosis) did not differ across groups and ranged from 2 to 12 years. The number of prior traumas reported by each group was not significantly different and ranged from 1 to 9.

Subjects ranged from 29 to 55 years of age ($M = 39.9 \pm 7.4$). Cancer mothers were significantly older than controls [$F(2,25) = 9.22, p = .001$]. The greatest age difference was between No PTSS ($M = 47 \pm 6.0$) and Control ($M = 34.44 \pm 5.22$), with PTSS mothers in between ($M = 40.00 \pm 6.2$). Age was retained as a covariate for all analyses.

Subjects identified themselves as Caucasian (64%), Asian (8%), Latina (8%), African American (8%) or “other” (12%). Groups did not differ on the basis of race/ethnicity, economic status (low-middle-high income), or health behaviors. Subjects reported for the past 24 h a range of 0-60 min of aerobic exercise, consumption of 0-2 alcoholic drinks and 0-5 cups of coffee, 6-12 h of sleep, 0-1 cigarettes and no drug use.

3.2. Subjective units of distress

As seen in Fig. 1, the MAT and Imagery Stressors were effective in increasing SUD anxiety compared to neutral phases (i.e., Baseline and Recovery phases). A repeated measures ANOVA confirmed the effect of phase [$F(1,21) = 36.24, p < .001$] and a group $\times$ phase interaction [$F(2,21) = 4.82, p = .02$]. The overall group effect was significant [$F(2,21) = 9.16, p < .001$] and contrasts indicated the PTSS group reported more distress than either No PTSS ($p = .008$) or control groups ($p < .001$), who did not differ ($p = .33$). Parameter estimates further showed these group differences were significant at MAT and Imagery stressor phases and Recovery 1; groups did not differ significantly at baseline ($p = .10$) or final recovery ($p = .15$). The interaction suggests that although all mothers responded with anxiety to the stressor challenges, PTSS mothers reacted more strongly.

3.3. Immune outcomes

Greater alcohol consumption [$F(1,19) = 3.05, p = .10$] and better sleep quality [$F(1,19) = 29.64, p < .001$] were associated with lower percentage total lymphocytes. These potential health modifiers were therefore retained in all analyses. Age was significantly associated with NK cells but not with any other immune outcome. Nonetheless, because of group age differences, age was included in all analyses. Coffee consumption and minutes of aerobic exercise were associated with NK cell levels and thus were included in all NK analyses. See below for F and p values and the direction of covariate impact for each immune outcome.

3.3.1. Resting values—Table 2 shows resting lymphocyte distributions (numerative and percentage of total) as a function of group. Repeated measures ANCOVA of total number of lymphocytes over phases indicated no group [$F(1,22) = .32, p = .58$] or age [$F(1,22) < 1, p = .94$] effects overall or at any phase. Group and phase patterns were similar for numerative and

percentage CD4+, CD8+, NK and total lymphocytes. Thus, we report only percentage data here.

3.3.2. CD4+:CD8+ ratio—After controlling for age, sleep quality, and alcohol consumption, repeated measures analyses indicated trends for Order 4 effect of phase [$F(1,19) = 3.71, p = .07$] and group $\times$ phase interaction [$F(2,19) = 2.69, p = .09$]. Also, there was a significant group effect [$F(2,19) = 5.88, p = .01$]. See Fig. 2. Contrasts indicated the group effect was due to significant differences between PTSS versus controls ($p = .04$) and PTSS versus No PTSS ($p = .006$), but not between No PTSS and controls ($p = .35$). Parameter estimates for group differences at each phase showed PTSS CD4+:CD8+ ratios were marginally lower than controls at baseline, Recovery 1 and Recovery Final [$p = .06, .08, .10$, respectively] and significantly lower than controls at the MAT and Imagery Stressor phases [$p = .03, .02$, respectively]. There were no significant differences between No PTSS and controls, overall or at any time point. The statistical trends for phase and group $\times$ phase interaction are likely due to a response pattern most consistent with SUD anxiety changes across phases among controls (see Fig. 1), little CD4+CD8+ ratio change for the No PTSS group, and moderate reactivity across phases among PTSS mothers.

3.3.3. NK cells—After controlling for age, alcohol and coffee consumption, sleep quality, and minutes of aerobic exercise, the repeated measures ANCOVA of NK cells revealed no significant effect of phase [$F(1,18) = .64, p = .44$], a trend for a group $\times$ phase interaction [$F(2,19) = 3.24, p = .06$] and no overall group effect [$F(2,19) = 1.41, p = .27$]. See Fig. 3. Contrasts confirmed no significant group differences overall, but parameter estimates showed after the MAT Stressor controls were significantly higher than PTSS ($p = .04$) but not the No PTSS group ($p = .16$). Groups were not different at baseline or at any other phase. Visual inspection of Fig. 3 suggests the statistical trend for a group $\times$ phase interaction reflects the reduced MAT Stressor reactivity among PTSS mothers relative to controls. Analysis of MAT Stressor change scores confirmed significantly greater reactivity among controls ($M = 5.05, SE = 1.0$) compared to PTSS mothers ($M = 1.93, SE = 0.8$) ($p = .03$) but not the No PTSS group ($M = 2.31, SE = 1.2$) ($p = .14$).

3.3.4. Impact of covariates—Age and sleep quality were not significantly associated with CD4+ or CD8+ levels, but alcohol consumption (0, 1 or 2 drinks) was associated with significantly higher CD4+ [$F(1,19) = 5.02, p = .04$] and lower CD8+ levels [$F(1,19) = 5.91, p = .03$]. Neither alcohol nor coffee consumption was significantly associated with NK levels [$F(1,17) = 2.07, p = .17$; $F(1,17) = 2.89, p = .11$, respectively]. In contrast, older age [$F(1,17) = 5.68, p = .03$], better sleep quality [$F(1,17) = 4.47, p = .05$], and less aerobic activity [$F(1,17) = 8.49, p = .01$] were significantly associated with higher NK levels in this sample.

3.4. Correlates of immune indicators

3.4.1. PTSD symptoms—To examine whether group differences in baseline and change score immune indicators were related to PTSS, correlations were conducted. As shown in Table 3, the greater the number and severity of symptoms, the higher the CD4+ and the lower the CD8+ levels at rest. Symptoms were unrelated to NK levels at rest, but were strongly associated with less NK change following the MAT stressor. Symptoms were unrelated to CD4+ and CD8+ + MAT stressor changes and to all immune changes in response to the Imagery stressor.

3.4.2. SUD anxiety—To determine the influence of baseline anxiety on immune outcomes, all ANCOVA analyses were repeated with resting SUD scores as a covariate. Immune group and phase effects were unchanged. Multivariate linear regression analyses with backward elimination were conducted to assess the relative contribution of SUD changes in response to the MAT stressor as a predictor of MAT immune changes. The best model for NK cells [F

(2,24) = 7.67, $p = .003$, $R^2 = .41$] included aerobic activity ($\beta = -.05$, $p = .09$) and number of PTSS symptoms ($\beta = -.33$, $p = .002$). Neither MAT SUD change nor PTSS group status was predictive of NK change in any model. For CD4+, the best model [$F(3,23) = 3.68$, $p = .03$, $R^2 = .36$] included age ($\beta = -.10$, $p = .13$), PTSS status ($\beta = 1.21$, $p = .06$) and MAT SUD change ($\beta = -.59$, $p = -.02$). Number of PTSS symptoms was not predictive of CD4+ change in any model. For CD8+, the best model [$F(4,23) = 4.99$, $p = .006$, $R^2 = .51$] included alcohol ($\beta = 1.61$, $p = .11$), PTSS status ($\beta = 2.10$, $p = .02$) MAT SUD change ($\beta = .91$, $p < .001$) and number of PTSS symptoms ($\beta = .28$, $p = .08$). Thus, subjective distress and PTSS status appear to be independent contributors to CD4+ and CD8+ immune changes in response to a stressor whereas only PTSS status predicts NK changes.

3.4.3. Symptoms of depression—BDI scores did not correlate with baseline immune levels or change scores for any immune indicator.

3.5. Discussion

This first study of lymphocyte distribution under psychological challenge among mothers of child cancer survivors indicates PTSD symptoms are associated with tonic elevation of CD4+:CD8+ ratios and blunted NK reactivity to generic challenge. Confirming prediction of group differences at rest, the PTSS group had higher percentage CD4+ and lower CD8+ levels than non-symptomatic women. Contrary to expectations, only cancer mothers with PTSS showed evidence for blunted reactivity. Also unlike predictions, reactivity to challenge was greatest to the non-social MAT stressor compared to the personalized challenge for all groups.

PTSS effects were evident in several ways. PTSS mothers had a significantly smaller change in NK cells following the MAT challenge than controls. Resting CD4+ and CD8+ levels and NK MAT change scores were all significantly correlated with number of PTSS symptoms, providing further evidence of tonic and phasic immune differences associated with PTSS. Multivariate regression results showing the separate predictive value of changes in subjective distress and PTSS status in estimating CD4+ and CD8+, but not NK MAT changes, indicates PTSS immune differences cannot be attributed to subjective distress alone.

For all groups, the direction of lymphocyte distribution in response to stressor challenge (percentage increases in NK and CD8+, decreases in CD4+) was consistent with previous studies (Cacioppo et al., 1998; Mills et al., 1995; Pike et al., 1997; Strauman et al., 1993; Valdimarsdottir et al., 2002). Likewise, PTSS-related immune effects found here provide replication evidence for some previous studies. Elevated resting CD4+ levels in PTSS mothers replicate findings of male combat veterans with chronic partial PTSD (Boscarino and Chang, 1999). Low resting levels of CD8+ are in keeping with some PTSS findings (Ironson et al., 1997), but not others (Boscarino and Chang, 1999; Laudenslager et al., 1998; Wilson et al., 1999). The absence of PTSS baseline differences in NK cells is in contrast to data from a natural disaster sample (Ironson et al., 1997).

Effects for cancer mothers as a chronic stress group are equivocal. Only PTSS mothers showed significantly reduced NK reactivity consistent with previous laboratory challenge studies among individuals high in life stress, daily hassles or tendency to worry (Benschop et al., 1994; Pike et al., 1997; Segerstrom et al., 1999). Although No PTSS mothers appeared to have blunted CD4+:CD8+ ratio reactivity (all phases) and smaller NK MAT Stressor change scores relative to controls, these differences were not statistically significant. Small sample size may be one explanation for the failure to detect significant differences. Alternatively, the absence of No PTSS differences may reflect exceptional resilience among mothers who do not develop long-term PTSS despite exposure to acute and chronic stress associated with their child's cancer. Past laboratory challenge studies did not assess PTSS, thus it is also possible these symptoms mediated reactivity effects of previous research. Further research will be necessary

to clarify whether PTSS confers immune changes separate from those associated with a history of chronic stress.

Group effects found here were not limited to individualized stressors, but emerged under generic challenge. The absence of strong reactivity to the Imagery Stressor in any group was unexpected based on previous data indicating interpersonal stressors have greater impact on lymphocyte distribution than non-social stressors (Herbert and Cohen, 1993). The significant rise in SUD anxiety during the Imagery Stressor as well as the MAT Stressor indicates subjects were as distressed by the Imagery as by the MAT Stressor. Thus, blunted lymphocyte distribution does not appear to be due to failure of the Imagery Stressor to induce distress. Although groups returned to baseline levels after the MAT Stressor, the inability to mount a significant change in lymphocyte distribution after the Imagery Stressor suggests reactivity may be temporarily blocked or reduced by an earlier stressor. Alternatively, circadian rhythm of immune measures may have influenced immune response strengths later in the laboratory session as compared to earlier. However, if that were the case, one would expect a rising pattern in CD4+, CD8+ and NK cell counts during the period coinciding with the lab session (approximately 6-8 p.m.), not stasis or a decline (Kronfol et al., 1997). Replication with generic and individualized stressors counterbalanced for order and separated by days would clarify the relative strength of group effects under each stressor condition.

Additional limitations of this study should be addressed in future research. First, this work must be replicated with a larger sample size to increase confidence in patterns found here. Also, whereas health behaviors such as sleep quality, exercise, use of alcohol, and coffee were statistically controlled here, a larger sample would allow for statistical modeling that could determine a mediating or moderating role for these factors, as has been done for health behaviors and depression (Miller et al., 1999). Second, blunted reductions in NK cell percentages following laboratory stress recently reported in females in the luteal phase of menses (Pehlivanoglu et al., 2001) suggests menses stage may influence outcomes. Finally, recent evidence for dampened NK cytotoxicity in the face of increased cell number (Pike et al., 1997; Strauman et al., 1993) emphasizes the importance of multi-method designs to clarify the mechanisms of immune change.

The present findings are preliminary and should be viewed as a guide for future research. Nonetheless, these data indicate posttraumatic stress symptoms are associated with changes in tonic lymphocyte distribution and immune responsivity to psychological challenge. In addition, these changes may be distinct from patterns associated with subjective distress more generally. Further research will be needed to assess the extent to which differential lymphocyte distribution effects found in the laboratory have direct implications for health in the natural environment.

Acknowledgments

The first author thanks Michael Irwin for his excellent feedback on an earlier draft, the mothers of child cancer survivors at UCLA for their generous participation; and finally, research assistants Irene Choi, Mark Power, Jennifer Bennett, Marleen Castaneda, and students too numerous to name for their diligent efforts in supporting this work.

References

- Beck AT, Steer RA. Internal consistencies of the original and revised Beck Depression Inventory. *J. Clin. Psychol* 1984;40:1365–1367. [PubMed: 6511949]
- Benschop RJ, Brosschot JF, Godaert GL, De Smet MB, Geenen R, Olff M, Heijnen CJ, Ballieux RE. Chronic stress affects immunologic but not cardiovascular responsiveness to acute psychological stress in humans. *Am. J. Physiol* 1994;266:R75–R80. [PubMed: 8304558]

- Boscarino JA, Chang J. Higher abnormal leukocyte and lymphocyte counts 20 years after exposure to severe stress: Research and clinical implications. *Psychosom. Med* 1999;61:378–386. [PubMed: 10367620]
- Cacioppo JT, Berntson GG, Malarkey WB, Kiecolt-Glaser JK, Sheridan JF, Poehlmann KM, Burleson MH, Ernst JM, Hawkey LC, Glaser R. Autonomic, neuroendocrine, and immune responses to psychological stress: the reactivity hypothesis. *Ann. N. Y. Acad. Sci* 1998;840:664–673. [PubMed: 9629293]
- DSM-IV. Diagnostic and Statistical Manual of Mental Disorders. fourth ed.. American Psychiatric Association; Washington, DC: 1994.
- Foa, EB. Posttraumatic Stress Diagnostic Scale (PDS) Manual. National Computer System; Minneapolis: 1995.
- Foa EB, Cashman L, Jaycox L, Perry K. The validation of a self-report measure of posttraumatic stress disorder: the Posttraumatic Diagnostic Scale. *Psychol. Assessment* 1997;9:445–451.
- Glover D, Poland R. Urinary cortisol and catecholamines in mothers of child cancer survivors with and without PTSD. *Psychoneuroendocrinology* 2002;27:805–819. [PubMed: 12183216]
- Glover DA, Powers MB, Bergman L, Smits JAJ, Telch MJ, Stuber M. Urinary dopamine and turn bias in traumatized women with and without PTSD symptoms. *Behav. Brain Res* 2003;144(12):137–141. [PubMed: 12946604]
- Hawk L, Dougall A, Ursano RJ, Baum A. Urinary catecholamines and cortisol in recent-onset posttraumatic stress disorder after motor vehicle accidents. *Psychosom. Med* 2000;62:423–434. [PubMed: 10845356]
- Herbert TB, Cohen S. Stress and immunity in humans: a meta-analytic review. *Psychosom. Med* 1993;55:364–379. [PubMed: 8416086]
- Ironson G, Wynings C, Schneiderman N, Baum A, Rodriguez M, Greenwood D. Posttraumatic stress symptoms, intrusive thoughts, loss, and immune function after Hurricane Andrew. *Psychosom. Med* 1997;59:128–141. [PubMed: 9088048]
- Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Psychoneuroimmunology and psychosomatic medicine: back to the future. *Psychosom. Med* 2002;64:15–28. [PubMed: 11818582]
- Kronfol Z, Nair M, Zhang Q, Hill EE, Brown MB. Circadian immune measures in healthy volunteers: relationship to hypothalamic-pituitary-adrenal axis hormones and sympathetic neurotransmitters. *Psychosom. Med* 1997;59:42–50. [PubMed: 9021865]
- Laudenslager ML, Aasal R, Adler L, Berger CL, Montgomery PT, Sandberg E. Elevated cytotoxicity in combat veterans with long-term post-traumatic stress disorder: preliminary observations. *Brain Behav. Immun* 1998;12:74–79. [PubMed: 9570863]
- Miller GE, Cohen S, Herbert TB. Pathways linking major depression and immunity in ambulatory female patients. *Psychosom. Med* 1999;61:850–860. [PubMed: 10593638]
- Mills PJ, Soraya L, Dimsdale JE. Temporal stability of acute stressor-induced changes in cellular immunity. *Int. J. Psychophysiol* 1995;19:287–290. [PubMed: 7558995]
- Pehlivanoglu B, Balkanci ZD, Ridvanagaoglu AY, Durmazlar N, Ozturk G, Erbas D. Impact of stress, gender and menstrual cycle on immune system: possible role of nitric oxide. *Arch. Physiol. Biochem* 2001;109:383–387. [PubMed: 11935378]
- Pike JL, Smith TL, Hauger RL, Nicassio PM, Patterson TL, McClintick J. Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in humans. *Psychosom. Med* 1997;59:447–457. [PubMed: 9251165]
- Segerstrom SC, Glover DA, Craske MG, Fahey JL. Worry affects the immune response to phobic fear. *Brain Behav. Immun* 1999;13:80–92. [PubMed: 10373274]
- Shalev AY, Orr SP, Pitman RK. Psychophysiologic assessment of traumatic imagery in Israeli civilian patients with posttraumatic stress disorder. *Am. J. Psychiatry* 1993;150:620–624. [PubMed: 8465880]
- Strauman TJ, Lemieux AM, Coe CL. Self-discrepancies and natural killer cell activity: the influence of negative psychological situations on stress physiology. *J. Pers. Soc. Psychol* 1993;6:1042–1052. [PubMed: 8326467]

- Stuber MI, Kazak AE, Meeske K, Barakat L. Is posttraumatic stress a viable model for understanding responses to childhood cancer? *Child Adolescent Psychiatric Clinics North America* 1998;7:169–182.
- Stuber ML, Christakis DA, Houskamp B, Kazak AE. Posttrauma symptoms in childhood leukemia survivors and their parents. *Psychosomatics* 1996;37:254–261. [PubMed: 8849502]
- Valdimarsdottir HB, Zakowski SG, Gerin W, Mamakos J, Pickering T, Bovbjerg DH. Heightened psychobiological reactivity to laboratory stressors in healthy women at familial risk for breast cancer. *J. Behav. Med* 2002;25:51–65. [PubMed: 11845558]
- Wilson SN, van der Kolk B, Burbridge J, Fisler R, Kradin R. Phenotype of blood lymphocytes in PTSD suggests chronic immune activation. *Psychosomatics* 1999;40:222–225. [PubMed: 10341534]

Fig. 1.

Mean (and SE) SUD rating across phases. *Note:* A procedural error resulted in no recorded SUD rating for one control subject.

Fig. 2.
Mean (SE) CD4+:CD8+ Ratio across phases. Covariates: age, alcohol, and sleep.

Fig. 3. Mean (SE) NK cells across phases. Covariates: age, alcohol, coffee, aerobics, and sleep.

Table 1

Mean (and SD) number of prior traumas, PTSD symptoms and symptom severity as a function of group as assessed by PDS (Foa, 1995)

	PTSS (<i>n</i> = 9)	No PTSS (<i>n</i> = 7)	Controls (<i>n</i> = 9)
Prior trauma			
Number of traumas *	3.1 (1.1)	4.0 (2.4)	2.0 (1.1)
Range	1–4	2–9	1–4
PTSD symptoms			
Symptom severity **	24.00 (10.9)	6.43 (2.9)	4.33 (4.4)
Number of symptoms **	11.89 (2.2)	4.43 (1.6)	3.44 (3.0)

* $p = .05$.

** $p < .001$.

Table 2

Resting mean (and SD) lymphocyte distributions (numerative and percentage of total) as a function of group

		PTSS (<i>n</i> = 9)	No PTSS (<i>n</i> = 7)	Controls (<i>n</i> = 9)
CD4+	#	1433 (283)	972 (315)	1148 (378)
	%	53.33 (6.7)	47.71 (10.1)	42.78 (4.1)
CD8+	#	733 (128)	688 (106)	930 (424)
	%	27.67 (5.2)	34.14 (7.2)	33.33 (4.9)
NK	#	167 (61)	158 (95)	204 (69)
	%	6.22 (2.1)	7.43 (4.2)	7.67 (3.1)
Lymphocyte	#	2674 (307)	2053 (294)	2657 (764)
	%	37.00 (7.5)	35.71 (5.5)	38.22 (5.7)

Table 3

Bivariate correlations between PTSD symptoms and immune outcomes at rest and following stressor phases

<i>N</i> = 25	PTSD symptoms	
	Number	Severity
At rest		
CD4+	.41 *	.30
CD8+	-.40 *	-.34
NK	-.14	-.21
CD4:8 Ratio	.41 *	.38
MAT change		
CD4+	.12	.07
CD8+	.11	.19
NK	-.57 **	-.51 **
CD4:8 Ratio	-.08	-.11

*
p < .05.**
p < .01.