EMPirical contributions

Chronic Stress, Social Support, and Persistent Alterations in the Natural Killer Cell Response to Cytokines in Older Adults

Brian A. Esterling, Janice K. Kiecolt-Glaser, Joy C. Bodnar, and Ronald Glaser

To address the long-term physiological consequences of chronic stressors, 14 continuing or current family caregivers of Alzheimer’s disease (AD) patients, 17 former AD caregivers, and 31 control subjects were compared. Continuing and former caregivers did not differ on depressive symptomatology or perceived stress; both groups were significantly more depressed and stressed than controls. Furthermore, continuing and former caregivers did not differ in the response of NK cells in vitro to recombinant interferon-γ and recombinant interleukin-2, and both groups had a significantly poorer response to these cytokines than controls. The physiological and psychological consequences of chronic stressors may persist well beyond the cessation of the actual stressor.

Key words: stress, caregivers, Alzheimer’s, cytokines, NK cells

Acute and chronic stressors may have different behavioral and physiological consequences (Baum, Cohen, & Hall, 1993). When stressful events are brief or clearly time limited, psychological distress usually subsides after the event ends (Baum, 1987). However, when stressor exposure is prolonged or traumatic, longer term disturbances such as cognitive intrusion and behavioral avoidance, sleep problems, anxiety, depression, heightened catecholamine levels, and immune down regulation may persist after the event has ended (Baum et al., 1993; McKinnon, Weisse, Reynolds, Bowles, & Baum, 1989).

Research on disasters suggests that long-term changes in affect, stress-related behavior, physiological functioning, and mental health may occur (Baum et al., 1993). For example, even 6 years after the nuclear accident at Three Mile Island (TMI), a subset of individuals living near the damaged nuclear reactor exhibited continued cognitive intrusion and behavioral avoidance, suggesting that distress can persist even after a chronic stressor has objectively ended (Baum, 1990). Thus, the intensity and duration of the stressor are not the only determinants of chronic stress reactions (Davidson & Baum, 1993).

The mental health consequences of one chronic stressor, caregiving for a family member with Alzheimer’s disease, have been well documented (Light & Lebowitz, 1989; Zarit, Orr, & Zarit, 1985). Convergent data from several laboratories suggest that a high proportion of dementia family caregivers develop clinically significant anxiety and depressive symptomatology (Light & Lebowitz, 1989), including many older adults who had no prior history of any mood disorder (Dura, Stuckenberg, & Kiecolt-Glaser, 1990, 1991). In contrast, few studies have addressed the adaptation of caregivers after bereavement. Specifically, does general well-being return to precaregiving levels once the stresses of caregiving are alleviated, or does the elevated depressive and anxiety symptomatology persist well into the postcaregiving years? Recent data from our laboratory suggest that caregivers’ depressive symptomatology does not change significantly after the Alzheimer patient’s death; even an average of 2 years after the death of the Alzheimer’s disease patient, caregivers continued to exhibit significantly more depressive symptoms and syndromal depressive disorders than their noncaregiving peers (Bodnar & Kiecolt-Glaser, in press). In fact, depression did not differ between continuing and bereaved caregivers.

Caregivers’ persistent depressive symptomatology is different from “normal” bereavement. Although most studies show a higher incidence of depression and anxiety in widows and widowers within the first several months after bereavement compared with nonbereaved controls, group differences are generally not significant in follow-up data collected 1 to 2 years later (Harlow, Goldberg, & Comstock, 1991; Lund, Caserta, & Dimond, 1989; Thompson, Gallagher-Thompson, Futterman, Gilewski, & Peterson, 1991). However, in keeping with Baum’s (1990) TMI data, recollections of the stressor (i.e., caregiving or memories of the spouse) can sustain chronic stress after caregiving ends. Bereaved caregivers who ruminated more about caregiving reported more depression, perceived stress, and greater social isolation compared with bereaved caregivers who ruminated less about their former caregiving role (Bodnar & Kiecolt-Glaser, in press). Longitudinal data from our laboratory suggest that caregiver

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This study was supported by National Institute of Mental Health Grant R37 MH42096.

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stress is associated with poorer immune and health outcomes (Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991; Kiecolt-Glaser & Glaser, 1989); compared with demographically matched noncaring control subjects, peripheral blood leukocytes (PBLs) obtained from Alzheimer’s disease patients’ caregivers had significantly reduced cellular proliferative responses to concanavalin A and phytohemagglutinin. In addition, caregivers had higher antibody titers to latent Epstein-Barr virus than matched control subjects, reflecting a down regulation of cellular immunity resulting in some loss of control over the replication of the latent virus (Glaser & Kiecolt-Glaser, in press; Rickinson et al., 1981). Caregivers report fewer important personal relationships, less closeness in their relationships, and less emotional and tangible support than their noncaring age peers (Kiecolt-Glaser et al., 1991). Moreover, those caregivers who reported lower social support at intake into the study showed greater immunological down regulation a year later. Caregivers also reported experiencing significantly more days of infectious illness, primarily upper respiratory tract infections, compared with their noncaring community counterparts (Kiecolt-Glaser et al., 1991).

Other data also suggest that chronic stressors may alter immune function. For example, McKinnon et al. (1989) found significantly fewer B lymphocytes, T-suppressor/cytotoxic lymphocytes, and natural killer (NK) cells in TMI-area residents compared with controls who lived elsewhere. Furthermore, TMI-area residents had higher levels of epinephrine and cortisol and higher antibody titers to two latent herpes viruses (herpes simplex virus type 1 and cytomegalovirus) compared with control subjects; again, these data suggest a down regulation of the cellular immune response, because cellular immunity plays a key role in controlling latent herpesvirus expression (Glaser & Kiecolt-Glaser, in press; Rickinson et al., 1981).

The NK cell differences between TMI residents and controls could have health implications, because NK cells play an important role in a variety of immune functions, including defense against viral infections (Welsh, 1986) and surveillance of tumor cells (Herberman & Ortaldo, 1981). NK cell cytotoxicity can be down regulated by stress, presumably through neuroendocrine mechanisms (Glaser, Rice, Speicher, Stout, & Kiecolt-Glaser, 1986; Irwin, Daniels, Bloom, Smith, & Weiner, 1987; Levy, Herberman, Lippman, & d’Angelo, 1987). Cytokines such as recombinant interferon-gamma (rIFN-γ) and recombinant interleukin-2 (rIL-2) enhance NK cell and lymphocyte-activated killer (LAK) cell cytotoxicity, respectively, in vitro (Herberman & Ortaldo, 1981), and there is evidence that stress can modulate IFN-γ and IL-2 synthesis by mitogen-treated PBLs (Dobin, Harth, McCaın, Martin, & Cousin, 1991; Glaser et al., 1986). Furthermore, a group intervention was associated with increases in IFN-alpha augmented NK cell activity in malignant melanoma patients, and this effect paralleled decreases in anxiety and depression (Fawzy et al., 1990). In this study we hypothesized that continuing and bereaved caregivers would show a poorer NK/LAK cell response to rIFN-γ and rIL-2 stimulation in vitro than noncaring controls.

Method

Subjects

Subject recruitment. Subjects for this study were part of a larger, longitudinal study of caregiver stress, health, and immune function (see Dura et al., 1990; Kiecolt-Glaser et al., 1991). Subjects were recruited from multiple sources in the Columbus, Ohio, area—including local dementia evaluation centers in area hospitals, neurologists’ referrals, the city’s Alzheimer’s Association (AA) support groups, the monthly AA newsletter, respite care programs, and governmental caregiver support programs. At intake into the study, all caregivers were caring for relatives with Alzheimer’s disease or a related dementing illness. For study entry, caregivers had to be providing 5 or more hours of care per week.

Control subjects were recruited through newspaper advertisements, senior citizen centers, area newsletters, church groups, university alumni publications, and referrals from other participants; potential control subjects who reported any caregiving activities were excluded. All subjects gave written informed consent for participation after the procedures were explained.

Subject characteristics. The first 62 subjects scheduled for their fifth annual appointment for the larger study constituted the sample for this study; the 62 subjects included 14 continuing caregivers, 17 bereaved caregivers, and 31 comparison group subjects. The majority (94%) were White. The average annual family income, between $20,000 and $30,000, did not differ among the three groups. The majority of subjects (62%, 78%, and 71% for continuing, bereaved, and control subjects, respectively) had at least some college education. There were no significant differences between the continuing and bereaved caregivers and controls in age (F < 1); education (F < 1); race χ²(2) = 2.78; or gender, χ²(2) = 0.03. All subjects were paid $30 for their annual participation in the study.

Continuing caregivers were defined as those caregivers who remained active in the caregiving role throughout the 5 years of observation. The continuing caregiver group included 5 men and 9 women with an average age of 68.0 years (SEM = 3.21). Continuing caregivers had been providing care for an average of 62.53 months (SEM = 16.00) at intake (i.e., Year 1) and thus had been providing care for an average of 10.21 years. They reported spending an average of 5.08 hr/day in caregiving activities, with approximately half (54%) of the patients living with the caregiver and the rest (46%) residing in nursing homes.

Bereaved caregivers, those whose relative had died in the interval between intake and Year 5, included 5 men and 12 women with an average age of 72.3 years (SEM = 2.06). The average length of time since the death of their impaired relative was 26.57 months (SEM = 3.09). At intake, bereaved caregivers had been providing care for an average of 80.5 months (SEM = 19.14), and the majority (72.2%) were providing this care at home. It is not surprising that the bereaved group had been providing care for significantly more years than the continuing caregiver group at Year 1, because bereaved caregivers reported spending an average of 6.97 hr per day (SEM = 0.82) in caregiving activities before their relative’s death in Year 1.

The control group comprised 9 men and 22 women with an average age of 70.9 years (SEM = 1.82). Comparison group subjects did not have any kind of caregiving responsibilities.

Self-Report Distress Measures

Depression. The severity of depressive symptoms in subjects was assessed using the Hamilton Depression Rating Scale (HDRS; Hamilton, 1967). The HDRS is a 24-item interviewer-rated measure of depression that provides information on depressive symptomatology.
for the week before the interview period. Interrater reliability, calculated annually for 10% of the sample using audiotapes, was \( r = .84 \), and the coefficient alpha for Year 5 was .74.

**Perceived stress.** The Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983) is a 14-item scale that assesses state perceptions of stress and measures the degree to which individuals appraise perceptions of daily life as unpredictable, uncontrollable, and overloading. Coefficient alphas for this assessment for Year 5 was .88.

**Impact of events.** The Impact of Events Scale (IES) measures intrusive and avoidance thinking in individuals after a specific stressful event (Horowit, Wilner, & Alvarez, 1979). Extensive use with posttraumatic stress disorder populations has provided normative data and established the scale's clinical utility (Arata, Saunders, & Kilpatrick, 1991; Weisenberg, Solomon, Schwarzwald, & Mikulincer, 1987). A modified form of the IES was used with bereaved caregivers to examine rumination specifically about their former caregiving situation. Bereaved subjects were asked to indicate the frequency of their intrusive thoughts and avoidance behaviors related to caregiving within the 7 days before their appointment.

**Social Support Interview**

The Social Support Interview (Fiore, Becker, & Coppel, 1983; Kiecolt-Glaser et al., 1991) asked subjects to "list the people in your life who are important to you, with whom you have contact, whether or not you like them," up to a total of 10. Subjects subsequently made independent ratings of the degree to which they perceived each of the relationships to be helpful and upsetting/troubling on a scale ranging from not at all (0) to extremely (6), with respect to both emotional support and tangible assistance. For each person named, subjects rated the frequency of contacts from daily (5) to less than monthly (1). Closeness was rated from not at all close (0) to extremely close (10).

**Health-Related Behaviors and Infectious Illness Assessments**

Potential immunomodulatory confounds such as general health status, medication use, nutritional status, caffeine intake, cigarettes smoked, sleep, and alcoholic drinks consumed were assessed. Nutritional status was assessed by measuring serum albumin levels as previously described (Kiecolt-Glaser et al., 1987). Self-report questionnaires assessed general health status, amount of caffeine and alcohol intake in the past 48 hours, smoking, and amount of vigorous physical exercise in the past week. In addition, major medications that could have significant modulatory effects on immunity were assessed. These included beta blockers, diuretics, and analgesics. No subjects had health problems with an immunological component (e.g., cancer or recent surgery) or were using other drugs that had obvious immunomodulatory effects.

To assess infectious illness episodes, we used the Health Review (Jenkins, Kraeger, Rose, & Hurst, 1980), a checklist of specific illness symptoms related to infectious disease (primarily upper respiratory illness). Validational evidence (Jenkins et al., 1980) showed that physicians' diagnoses were the same as those defined by a computer algorithm using Health Review symptom clusters in 77% of the cases examined. Moreover, all diagnostic differences were minor ones within the general category of acute respiratory illness.

Subjects were assessed at 3-month intervals, and these data were transformed to reflect illness in the last 12 months. Thus, Health Review data are reported for the previous 12-month period and yielded three variables related to total number of illnesses, length of illness episode, and number of visits to the doctor for infectious illness symptoms. We were interested in the presence or absence of an infectious illness episode, not specific diagnoses. Data from each Health Review interview were reviewed by our project nurse, who used preestablished International Classification of Disease—9 (ICD-9)-based criteria to decide whether a subject had an infectious illness. When necessary, follow-up telephone calls were made to collect additional information. The validity of the Health Review for diagnosing infectious illness is strongly supported by data from a previous study in which physicians provided an infectious illness diagnosis when subjects had also met our criteria (Kiecolt-Glaser et al., 1991).

Test-retest and interrater reliability were also high for the Health Review (Kiecolt-Glaser et al., 1991).

The Health Review was administered as an interview. Subjects were read the symptom list and were asked to indicate which symptoms occurred as part of an illness episode, as isolated symptoms, or as more chronic problems, with operational definitions provided for these categories. Subjects were also asked whether they saw a physician for the problem(s) and the number of days they were unable to perform their normal daily activities because of illness. To enhance subjects' recall of important events, we used a series of memory prompts from Bradburn, Raps, and Sowell (1987), for example, reminding subjects of their data from the last interview or highlighting important events that occurred in the time period, such as major holidays.

**Treatment of Peripheral Blood Leukocytes with rIL-2 and rIFN-γ**

To control for diurnal variation, blood samples were collected between 8:00 a.m. and 10:00 a.m. Peripheral blood leukocytes were isolated on Hypaque-Ficoll gradients, washed in calcium and magnesium-free, phosphate-buffered saline, and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 0.75% sodium bicarbonate, 2mM L-glutamine, and 25μg/ml gentamicin. Isolated PBLs were prepared at a concentration of 2.8 x 10^6 cells/ml, then seeded, 7.0 x 10^6 total cells/well in 3 replicate wells of 12-well plastic plates (Corning Corporation). Cells were then provided with either complete RPMI 1640 media alone, complete media supplemented with 60 units/ml rIL-2 (Genzyme Corporation), or complete media supplemented with 250 units/ml rIFN-γ (Genzyme Corporation). These concentrations were determined by standard dose-response relationships, in separate experiments in our laboratory, which provided the minimum dose needed to produce a maximal effect. Cell suspensions with cytokines were gently mixed and then incubated at 37 °C in an atmosphere of 5% CO₂ for 65 hr.

**NK Cell and Lymphokine-Activated Killer Cell Assay**

A microtiter ⁵¹Cr-release cytotoxicity assay was used to determine NK cell activity in both studies. The target cells used in the assay were K-562 cells, an NK-cell-sensitive myeloid cell line. After the 65-hr incubation with rIFN-γ or rIL-2, cells were washed 3 times in complete RPMI 1640 medium. Triplicate aliquots of PBLs, and target cells labeled overnight for 16 hr with ⁵¹Cr, were placed in wells of 96-well plates (Linbro, Hampton, Connecticut), resulting in effector to target cell ratios of 50:1, 25:1, and 12.5:1. In addition, triplicate wells with target cells and medium only and target cells and detergent (1% sodium dodecyl sulfate) were prepared to determine spontaneously released radioactivity and maximal lysis, respectively. Cell suspensions were centrifuged at 1,000 rpm (200 x g) for 5 min to bring the effector and target cells into contact. Cells were incubated at 37 °C in an atmosphere of 5% CO₂ for 5 hours. Following the 5 hour incubation, the plates were centrifuged at 1500 rpm (300 x g) for 5 min, 100 μl of each supernatant was collected, and counts per minute (CPM) were determined by means of a Beckman 9000 gamma counter. Results are...
Figure 1. The response of natural killer cells (±SEM) from continuing and bereaved caregivers and controls to 250 IU/ml rIFN-γ above RPMI 1640 media control values.

reported as the percentage lysis adjusted for media covariation using the formula:

\[
\text{Change in % lysis} = \frac{\text{Exp}_e \times CPM - \text{SR}_e \times CPM}{\text{MR}_e \times CPM - \text{SR}_e \times CPM} \times 100 - (\text{residualized } \beta) \\
\times \frac{\text{Exp}_m \times CPM - \text{SR}_m \times CPM}{\text{MR}_m \times CPM - \text{SR}_m \times CPM} \times 100
\]

\(\text{Exp}_e = \text{experimental CPM}_{\text{cytokine}}\)
\(\text{SR}_e = \text{spontaneous CPM}_{\text{cytokine}}\)
\(\text{MR}_e = \text{maximum CPM}_{\text{cytokine}}\)

\(\text{Residualized } \beta = \text{regressed media covariation coefficient}\)

\(\text{Exp}_m = \text{experimental CPM}_{\text{media}}\)
\(\text{SR}_m = \text{spontaneous CPM}_{\text{media}}\)
\(\text{MR}_m = \text{maximum CPM}_{\text{media}}\)

This procedure permitted the determination of the effectiveness of rIL-2 and rIFN-γ to stimulate LAK and NK cell activity, respectively, over baseline levels found in the standard NK cell cytotoxicity assay. The data are presented as percentage lysis rather than lytic units because the models for determining lytic unit values incorporate assumptions and methods of calculation that can result in inaccurate model-predicted cytotoxicity in comparison with actual observed cytotoxicity (Pollock, Zimmerman, Fuchshuber, & Lotzova, 1990).

Determination of the percentage of NK cells. PBLS were adsorbed with a monoclonal antibody (mAb) conjugated to fluorescein isothiocyanate (FITC) against the cell surface marker CD56 (Coulter Corporation), an NK cell surface marker. Briefly, 0.5 × 10^6 cells were incubated with the mAb for 30 min at 4 °C. Cells were then washed, fixed with paraformaldehyde, and analyzed using an EPICS C flow cytometer (Coulter Corporation). CD56+ cells have the morphology of large granular lymphocytes and mediate non-MHC-restricted lysis of K562 cells (Lanier, My Le, Civin, Loken, & Phillips, 1986).

Data Analyses

We used repeated measures multivariate analyses of variance (MANOVAs) to assess differences among continuing and bereaved caregivers and controls on NK/LAK cell cytotoxicity, social support dimensions, and Health Review variables. When significant main effects were found, post hoc comparisons were conducted using the Tukey test for pairwise comparisons. To control for varying numbers of NK cells used in the assay system, NK/LAK cell cytotoxicity was covaried with percentage NK cells. Additionally, a series of one-way analyses of variance (ANOVA) compared continuing and bereaved caregiver groups and control subjects on measures of perceived stress and depression. Pearson correlations were computed between self-reported health behaviors and NK/LAK cell cytotoxicity. In addition, when gender was added as a second between-subjects factor, neither the main effects of gender nor the Gender × Group interactions approached significance (Fs < 1).

Results

Effect of rIFN-γ and rIL-2 and on NK/LAK Cell Activity

To investigate the response of NK/LAK cells to cytokine stimulation, PBLS were incubated with either rIFN-γ or rIL-2 for 65 hr. Percentage lysis, adjusted for media covariation (i.e., residualized scores), was calculated for both rIFN-γ and rIL-2 stimulation. A 3 (group membership) × 3 (effector:target cell ratio) repeated measures MANOVA identified significant group differences across the different effectortarget cell ratios, F(2, 59) = 7.54, p < .001, with respect to rIFN-γ (Figure 1). Repeated measures planned-comparison contrasts showed that NK cells obtained from both continuing caregivers, F(1, 42) = 8.77, p < .01, and bereaved caregivers, F(1, 47) = 7.66, p < .01, had a significantly poorer response to 250 IU/ml rIFN-γ compared with controls. The continuing and bereaved caregivers did not differ in the NK response to rIFN-γ, F(1, 29) = 1.42, ns.

In investigating the LAK cell response to rIL-2, we found a significant Group × Dilution interaction for continuing caregivers, bereaved caregivers, and controls, F(4, 118) = 2.58, p < .05 (Figure 2). Analysis of simple main effects showed that continuing and bereaved caregivers did not differ from each other (Fs < 1), whereas both groups had a significantly poorer response to 60 IU/ml rIL-2 than control subjects at the 25:1 effectortarget cell ratio (p < .05).

NK cell cytotoxicity, independent of cytokine induction, was measured to examine group differences between the continuing and bereaved caregiver groups and controls. No significant group differences or interactions between group and effector target cell ratios were observed (Fs < 1; Figure 3). These latter data are consistent with results from Irwin et al. (1991).

Figure 2. The response of natural killer cells (±SEM) from continuing and bereaved caregivers and controls to 60 IU/ml rIL-2 above RPMI 1640 media control values.
Percentage NK Cells in the PBLs Used for Cytotoxicity Assays

We assessed the possibility that differences in the percentage of NK cells in the PBL samples might account for group differences in cytokine response. Mean cell fluorescence (MCF; a measure of receptor density on the cell surface) was also assessed. Neither percentage, \( F(2, 28) = 0.49, ns \), nor MCF, \( F(2, 28) = 2.96, ns \), differed among the continuing and bereaved caregiver groups and controls. As a further check, the group analyses used above were repeated using percentages of NK cells as a covered; the addition of the covariate did not alter the prior results.

Self-Report Distress Measures

Continuing and bereaved caregivers did not significantly differ in levels of depression or perceived stress (\( Fs < 1 \)). However, continuing and bereaved caregivers were significantly more stressed, \( F(2, 59) = 8.63, p < .001 \), \( M = 27.61, \text{SEM} = 2.21 \), and \( M = 24.83, \text{SEM} = 1.58 \), respectively) and depressed, \( F(2, 59) = 6.46, p < .001 \), \( M = 4.30, \text{SEM} = 1.48 \), and \( M = 5.72, \text{SEM} = 1.08 \), respectively) than control subjects (\( M = 19.12, \text{SEM} = 0.98 \), \( M = 1.61, \text{SEM} = 0.38 \), respectively).

Correlations were computed between NK/LAK response to rIFN-\( \gamma \) and rIL-2, total IES scores, and months bereaved for bereaved caregivers at the 25:1 effector:target cell ratio; because the distributions for IES scores and months bereaved were not normal, we computed Spearman correlations. Caregivers who had been bereaved for longer periods of time had higher levels of NK cell cytotoxicity after rIFN-\( \gamma \) stimulation (\( r = .48, p < .05 \)) and rIL-2 stimulation (\( r = .31, p < .10 \)). Although in the expected direction, correlations did not reach significance between total IES scores and rIFN-\( \gamma \) (\( r = -.34 \)) or rIL-2 (\( r = -.24 \)). However, the relatively small number of bereaved caregivers makes it difficult to draw inferences about possible mediational processes. IES scores were significantly correlated with both depression (\( r = .43, p < .01 \)), and perceived stress (\( r = .56, p < .01 \)). Finally, Pearson correlations were computed between perceived stress and depression scores and the NK/LAK response to rIFN-\( \gamma \) and rIL-2. The correlations between rIFN-\( \gamma \) and perceived stress (\( r = -.19 \)) and between rIFN-\( \gamma \) and depression (\( r = 0 \)) were not significant. Likewise, the correlations between rIL-2 and perceived stress (\( r = -.14 \)) and between rIL-2 and depression (\( r = -.24 \)) were nonsignificant.

Group Membership, Social Support, and Cytokine Response

Because it was possible that the differences in response to the two cytokines could be related to changes in NK cell integrity, the relationship between the response to rIFN-\( \gamma \) and rIL-2 in the total population was investigated. The Pearson correlation between subjects’ responses to the two cytokines was nonsignificant (\( r = .06 \)). Because of this low correlation, we used a median split to divide subjects for both rIFN-\( \gamma \) (10.0% increase over media control) and rIL-2 (48.5% increase over media control). This grouping created four groups that were classified as low responders to both cytokines (\( n = 15 \)), high responders to both cytokines (\( n = 16 \)), and those subjects who were either low responders to rIFN-\( \gamma \) and high responders to rIL-2 (\( n = 15 \)), or high responders to rIFN-\( \gamma \) and low responders to rIL-2 (\( n = 16 \)). Furthermore, because our bereavement and continuous caregiving groups did not differ on perceived stress, depressive symptomatology, or their NK cell response to either cytokine, continuing and bereaved caregivers were combined into a single, chronically stressed group. When comparing chronically stressed and nonstressed caregivers and their response to the two cytokines in a 4 (low response to rIFN-\( \gamma \) and rIL-2, high response to rIFN-\( \gamma \) and rIL-2, or differential response to both cytokines) \( \times 2 \) (caregivers vs. control) factorial design, significant differences in distributions were found, \( \chi^2(3) = 15.00, p < .001 \). Table 1 contains the distribution of subjects along these dimensions, with caregivers highly skewed toward the low rIFN-\( \gamma \)/low rIL-2 end, and controls highly skewed toward the high rIFN-\( \gamma \)/high rIL-2 end. Continuing and bereaved caregivers were equally distributed across these groups, \( \chi^2(1) = 1.43 \). Furthermore, age was not related to cytokine response classification (\( F < 1 \)).

Because the chronic stress of continuing caregiving or bereavement was related to both psychological (depressive symptoms and perceived stress) and immunological (a decrease in the response of NK cells to rIFN-\( \gamma \) and/or rIL-2) decrements, we examined differences in social support. Positive or upsetting emotional and tangible support, closeness, or number of supports did not differ between the combined caregiver group and controls (\( Fs < 1.2 \)). However, we and others have found that caregivers report less social support

<table>
<thead>
<tr>
<th>Group</th>
<th>Low rIFN-( \gamma ) rIL-2</th>
<th>High rIFN-( \gamma ) rIL-2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic stress</td>
<td>12 9 8 2</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3 6 8 14 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15 15 16 16 62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. NK = natural killer, rIFN = recombinant interferon, rIL-2 = recombinant interleukin-2.

![Figure 3](image-url) The response of natural killer cells (±SEM) from continuing and bereaved caregivers and controls.
Table 2
Pearson Correlations Between Health Behaviors and NK Cell Response to rIFN-γ and rIL-2

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Albumin</th>
<th>Caffeine</th>
<th>Cigarettes</th>
<th>Alcohol</th>
<th>Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>rIFN-γ</td>
<td>.11</td>
<td>.02</td>
<td>-.06</td>
<td>.24</td>
<td>-.13</td>
</tr>
<tr>
<td>rIL-2</td>
<td>.22</td>
<td>.15</td>
<td>.02</td>
<td>.08</td>
<td>.18</td>
</tr>
</tbody>
</table>

Note. NK = natural killer, rIFN = recombinant interferon, rIL-2 = recombinant interleukin-2.

than controls using data from larger samples (e.g., Kiecolt-Glaser et al., 1991; Light & Lebowitz, 1989).

When continuing and bereaved caregivers were divided into either low-cytokine responders (i.e., responding below the median to both rIFN-γ and rIL-2) or high-cytokine responders (i.e., responding above the median to either or both cytokines), low-cytokine responders reported less positive emotional and tangible supports (M = 38.67, SEM = 4.15), F(1, 29) = 4.31, p < .05, and rated less closeness in their relationships (M = 41.33, SEM = 5.00), F(1, 29) = 5.05, p < .05, compared with the high-cytokine responders (M = 56.42, SEM = 6.24, and M = 58.95, SEM = 5.34, respectively). Low- and high-cytokine responders did not differ on upsetting emotional and tangible supports or numbers of people in their network. Neither level of depressive symptoms (F < 1) nor perceived stress, F(1, 29) = 1.27, ns, differed between the low- and high-cytokine responders.

Health Data

Inclusion of the three Health Review variables in a single MANOVA showed poorer health in the continuing and bereaved caregivers compared with controls, F(6, 114) = 2.73, p < .01. Subsequent ANOVAs for each of the three variables showed that whereas continuing and bereaved caregivers did not differ from each other in their visits to the physician (M = 0.67, SEM = 0.24 vs. M = 0.66, SEM = 0.13), both caregiver groups visited their physician more than twice as often as controls for infectious illness symptoms (M = 0.24, SEM = 0.09), F(1, 60) = 7.88, p < .01. However, the total number of illnesses, F(1, 60) = 1.33, ns, as well as the length of their illness episodes (F < 1) did not differ between the groups.

We further investigated the continuing and bereaved caregivers to determine whether those caregivers who were low-cytokine responders also reported poorer health as defined by the Health Review. Inclusion of the three Health Review variables in a single MANOVA showed poorer health in the caregivers who were low- rather than high-cytokine responders, F(3, 27) = 3.90, p < .05. Subsequent ANOVAs showed that the low-cytokine responders visited their physician more than twice as often as the high-cytokine responders for infectious illness symptoms (M = 0.98, SEM = 0.19 vs. M = 0.42, SEM = 0.13), F(1, 29) = 5.73, p < .05. However, the total number of illnesses did not differ between the low- and high-cytokine responders (M = 1.22, SEM = 0.19 vs. M = 1.22, SEM = 0.28, F < 1), and the low- and high-cytokine responders did not differ in the length of their illness episodes (M = 4.01, SEM = 1.87 vs. M = 5.22, SEM = 2.20, F < 1).

The lower support of low responders may have made them more likely to seek support through their contacts with a physician; alternatively, they may have interpreted their illness as more severe and thus were more likely to seek medical treatment. Because base rates for illness episodes are generally low, a single year’s data may not provide sufficient information to assess associations between immune function and health changes.

Health Behaviors

Continuing and bereaved caregivers and controls did not differ on health-related behaviors (Table 2). Pearson correlations, computed between LAK/NK cell cytotoxicity and serum albumin levels, caffeine intake, cigarettes smoked, alcoholic drinks consumed, and sleep were nonsignificant. No significant differences were found with respect to use of beta blockers, diuretics, analgesics, or antihistamines between continuing and bereaved caregiver groups and controls; all groups reported minimal usage. Furthermore, no differences were found between any of these variables and the low- and high-cytokine responders.

Discussion

Although an average of over 2 years had elapsed since bereavement, continuing and bereaved caregivers did not differ on depressive symptomatology or perceived distress. Moreover, both groups were significantly more depressed and reported more stress than control subjects. Furthermore, continuing and bereaved caregivers did not differ in the ability of their NK cells to respond to rIFN-γ or rIL-2 in vitro, and both groups had a significantly poorer response than controls. These combined effects were independent of both the percentage of NK cells and mean cell florescence of the CD56 marker measured with a monoclonal antibody; the absolute number of NK cells was not determined. Although our bereaved caregivers could still be defined as compromised in their response to cytokine stimulation even 2 years postbereavement, there may be eventual recovery. Caregivers who had been bereaved for longer periods of time had significantly better responses to both cytokines.

In keeping with work from Irwin et al. (1991), we found no differences in NK cell cytotoxicity between either the continuing or bereaved caregiver groups or control subjects in the absence of cytokine stimulation. Although we previously found stress-related down regulation of the NK cell cytotoxic response (e.g., Kiecolt-Glaser et al., 1984), the current study differed from prior studies with respect to both the age of the subjects and the type of stressor (e.g., acute vs. chronic), suggesting that broader changes in NK cell cytotoxicity may be age or stressor dependent.

The NK cell cytotoxicity of the continuing and bereaved caregivers was highly skewed toward low responding (i.e., responding below the median for the population) after stimulation with either rIFN-γ or rIL-2. Caregivers who were low-NK cell cytotoxic responders to both cytokines reported significantly less positive social support and emotional closeness in their social contacts and more physician visits for infectious illness symptoms, compared with caregivers who were high responders to at least one cytokine.
These findings may have particular importance for older adults in the context of the glucocorticoid cascade hypothesis, which suggests that chronic stress may accelerate the process of immune down regulation associated with aging (Sapolsky, Krey, & McEwen, 1986). Thus, chronic stress could have longer term, potentially irreversible consequences. Specifically, changes in the ability of NK cells to respond to cytokines necessary for effective cell killing of appropriate tumor or virally infected cells may be found in chronically stressed older subjects and other groups at risk for malignant and infectious diseases.

Our major hypothesis regarding reduced NK cytotoxicity after cytokine stimulation in caregivers compared with control subjects was supported, suggesting that there may be a specific defect in the NK cell's ability to respond to such stimulatory cytokines. In keeping with evidence of other immunological down regulation in continuing caregivers, this study extends those findings to include caregivers who have been bereaved for over 2 years. In two previous studies from our laboratory, we found that examination stress modulated IFN-γ production by PBLs, concomitant with a decrease in NK cell cytotoxicity (Glaser, Rice, Sheridan, et al., 1987; Glaser, Rice, Speicher, Stout, & Keicoll-Glaser, 1986). Dobbin et al. (1991) also showed that IFN-γ could be suppressed after stressful events. Furthermore, stress-reduction strategies have been related to increases in IFN-alpha-stimulated NK cells in an assay system similar to the one used in the present study (Fawzy et al., 1990). Therefore, psychological stressors may reduce the production of cytokines that are important in stimulating NK cells to lyse target cells (e.g., micrometastases), in addition to reducing the NK cell's ability to use available cytokines, and thus possibly contributing to increased susceptibility to infectious illnesses.

References


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