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Evidence for a Shift in the Th-1 to Th-2 Cytokine Response Associated With Chronic Stress and Aging

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Background. *A* number of studies have shown that the chronic stress of caring for persons with dementia can have significant immunological consequences as demonstrated by the down-regulation/dysregulation of the cellular immune response.

Methods. Utilizing flow cytometry to measure the percentages and absolute numbers of CD-4^+ and CD-8^+ T lymphocytes producing the cytokines indicative of Th-1, Tc1 and Th-2, and Tc2 cells, we compared spousal caregivers and control subjects. The expression of interleukin-2 (IL-2), interferon gamma (IFN-γ), and interleukin-10 (IL-10) in the cytoplasm of CD-4^+ and CD-8^+ lymphocytes was assessed.

Results. Neither stress nor age was significantly related to the percentage or number of IFNγ^+/CD-8^+, IL-2^+/CD-8^+ cells, or IFNγ^+, IL-2^+, CD-4^+ cells. However, the percentage of IL-10^+ cells was higher in lymphocytes obtained from caregivers than control subjects. In addition, the significant interaction between stress and aging for IL-10^+/CD-4^+ and IL-10^+/CD-8^+ cells demonstrated that the difference between caregivers and control subjects was age dependent; the difference between caregivers and control subjects was substantially larger in younger individuals than in older individuals.

Conclusions. The data are consistent with previous reports on acute stress and suggest that there may also be a shift from a Th-1 to a Th-2 response associated with a chronic stressor such as caregiving. This shift could have implications for an individual's responses to pathogens.

THE chronic stress of caregiving for a family member with progressive dementia has significant immunological consequences. Kiecolt-Glaser and colleagues (1) found a poorer proliferative response of peripheral blood leukocytes (PBLs) to mitogens in caregivers compared with PBLs obtained from noncaregiving control subjects. Concomitant with changes in cellular immunity, caregivers reported significantly more days of infectious illness, primarily upper respiratory tract infections, and these reports were corroborated by physician data (1). McCann (2) reported that caregivers had poorer responses to delayed hypersensitivity skin testing than noncaregivers. Plasma levels of neuropeptide Y, a sympathetic neurotransmitter that is released during emotional stress and that may also modulate immunity (3), were significantly elevated in blood samples obtained from caregivers compared with nondepressed control subjects. We have reported that natural killer (NK) cells obtained from caregivers showed a poorer response to stimulation with recombinant gamma interferon (rIFN-γ) and recombinant interleukin-2 (rIL-2) than NK cells obtained from control subjects (4). Wound healing was also significantly delayed in caregivers compared with control subjects, and PBLs obtained from these subjects produced less interleukin-1 beta (IL- 1β) in vitro after stimulation with lipopolysaccharide (LPS) as compared with PBLs from control subjects (5).

In a further study, caregivers exhibited significant deficits relative to control subjects in both the antibody and virus-specific T-cell responses to an influenza virus vaccine (6). Caregivers were less likely than control subjects to show a fourfold increase in antibody titers to the trivalent flu vaccine 4 weeks post-vaccination, and they also showed a poorer virus-specific T-cell response in vitro. Additionally, PBLs from caregivers produced lower levels of IL-1β when stimulated with LPS. These data suggest that caregivers are potentially more vulnerable to the influenza virus and to other infectious agents than aged-matched control subjects (7). A recent study confirmed stress-related decrements in the antibody response to an influenza virus vaccination in caregivers of dementia patients (8). These vaccine data may provide a window on the body's response to pathogens, such as viruses or bacteria, and support the hypothesis that the immune modulation induced by psychological stressors is large enough to have health effects (9).

Importantly, preliminary data suggest that cessation of caregiving does not terminate risk; in the data collected thus far in our longitudinal study, former spousal caregivers have not differed significantly from "continuous" caregivers on cellular immune function after the death of the impaired spouse. In fact, spousal caregivers have continued to show immunological down-regulation for several years after bereavement (4).

For example, current and former caregivers did not differ from each other in the inability of their NK cells to respond to the stimulatory effects of rIFN-γ or rIL-2 (4), and both showed poorer responses to these cytokines than did control subjects. Similarly, the protective capacity of viral vaccines is dependent on their ability to induce both Immoral and cell-mediated immune responses (7); both were poorer in caregivers than in control subjects, and current caregivers did not differ from former caregivers (10).

In a recent study from our laboratory, we explored a possible mechanism underlying the stress-associated immune changes in caregivers by measuring lymphocyte growth hormone (GH) mRNA levels in lymphocytes. GH is an immune-enhancing hormone that may be important in modulating Immoral and cellular immune function (11). Using RT-PCR to measure GH mRNA levels in T- and B-cell populations, the level of GH mRNA was 50% less in cells obtained from caregivers compared with control subjects (11). These differences in GH mRNA levels in both B and T cells may be related to the poorer immune response to the influenza virus vaccine already discussed.

Different subpopulations of CD-4^+ T cells synthesize specific cytokines and have been designated Th-1 or Th-2 cells. Whereas there is some overlap between the patterns of cytokines synthesized by both these populations of lymphocytes, the evidence suggests that there is also specificity. Furthermore, the cytokine products of Th-1 and Th-2 cells tend to be mutually inhibitory. Additionally, CD-8^+ T-lymphocytes can secrete Th-1 as well as Th-2 cytokines; these cells are designated Tc1 and Tc2. Examples of Th-1- and Tc1-derived cytokines include IL-2 and IFN-γ. Th-2- and Tc2-derived cytokines include IL-4, IL-5, IL-6, and IL-10 (12).

Glucocorticoid hormones can profoundly affect cell-mediated immunity. Glucocorticoid hormones differentially modulate the expression of the cytokines IL-2 and IL-4, resulting in a polarized shift of T-cell responses to the Th-2 subset (13). The polarization of T-helper cell responses can be driven in vivo by the stress-induced elevation of endogenous glucocorticoid hormones, and this shift may play an important role in determining the host's response to a pathogen (14).

In this study we examined stress-associated immune alterations in caregivers by measuring the expression of cytokines synthesized by PBLs that help regulate and balance the cellular and humoral immune responses. Specifically, we measured the percentage and total number of $CD4^+$ and $CD8^+$ lymphocytes synthesizing IL-2, IL-10, and IFN-γ by flow cytometry. The data suggest that there may be a shift toward a Th-2 response associated with chronic stress and that this shift is related to age.

METHODS

Subject Characteristics

Subjects for this study were part of a longitudinal study of caregiver stress, health, and immune function (1,4,6). The longitudinal study includes ongoing recruitment into the cohort across years, and caregivers are followed after the death of the spouse. Subjects were recruited from multiple local sources, including dementia evaluation centers in area hospitals, neurologists' referrals, the city's Alzheimer's Association support groups and its newsletter, respite care programs, and governmental caregiver support programs. At the time of recruitment into the study, all caregivers were caring for a spouse with Alzheimer's disease or another progressive dementia and had to be providing five 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. The Ohio State University Biomedical Research Review Committee approved the project; all subjects gave written informed consent prior to participation.

The 76 subjects included 16 continuing caregivers, 16 former caregivers, and 44 control group subjects. There were no significant differences among the three groups in education (70% had at least a partial college education), race (82% Caucasian, 18% African American), or gender (71% were women). There was, however, a significant difference among groups with respect to age, $F(2,73) = 3.72$, $p < .05$; for control subjects, mean age = 69.89, $SD = 9.26$; for former caregivers, mean age = 77.00, SD = 9.51; for current caregivers, mean age = 71.69, $SD = 7.25$. All subjects were paid $40 for their annual participation in the study. Subjects were excluded if they reported diabetes, the use of anti-inflammatory medication or other medications with obvious immunological consequences, or immunologically related health problems (e.g., cancer, autoimmune disease, or recent surgery).

Continuing caregivers were defined as those who were still actively caregiving in the year these data were collected. Continuing caregivers had been providing care for an average of 104.11 months *(SEM* = 12.31). They reported spending an average of 7.60 h/d in caregiving activities. For former caregivers, an average of 2.57 years *(SEM* = 0.50) had elapsed since the death of their spouse, with a range of several months to 5 years.

Health-related behaviors were assessed when blood was drawn (15). Two questions assessed exercise (16). The 36-item Rand Health Survey (17) provided a nondisease specific measure of functioning with excellent normative data.

Measurement o f Cytoplasmic Cytokines Including IL-2, IL-10, and IFN-γ

Heparinized whole blood samples were obtained from each subject, and PBLs were obtained using routine procedures. To detect cells synthesizing IL-2, 4×10^6 PBLs were washed in phosphate-buffered saline (PBS). The PBLs were stimulated with 20 ng/ml phorbol-23 myristate 13-acetate (PMA) (Sigma, St. Louis, MO), 250 mg/ml ionomycin (Sigma), and 2 μM monensin (Sigma) in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) for 6 hours at 37°C. The cells were then placed in calcium- and magnesium-free Dulbecco's PBS (DPBS) containing l % heat-inactivated FBS and 0.1% sodium azide, pH adjusted between 7.4 and 7.6. The PBLs were adsorbed with either the surface marker IgG1 monoclonal antibody (MAb) conjugated to fluorescein isothiocyanate (FITC) (for control), or CD-4- FITC, CD-8- FITC, or CD-3- FITC MAbs (Pharmingen, San Diego, CA). The cells were washed and then resuspended in fixation buffer (4% paraformadehyde

in DPBS, with the pH adjusted between 7.4 and 7.6) for 20 minutes at 4°C, then washed twice with calcium- and magnesium-free DPBS, 1% heat-inactivated FBS, 0.1% sodium azide, 0.1% saponin, with the pH adjusted between 7.4 and 7.6. The cells were resuspended in permeabilization buffer containing 0.25 μg of a rat anti-human IL-2 MAb conjugated to phycoerythrin (PE) (Pharmingen). A block control sample was prepared by adding a rat anti-human IL-2 MAb with an excess of recombinant human IL-2 (Pharmingen) to a tube containing PBLs. The tubes were incubated for 30 minutes at 4°C, washed twice with staining buffer, pelleted, and resuspended in 100 μl staining buffer.

To detect IL-10^+ T cells, PBLs were incubated with 10 ng/ml LPS (Sigma) and 1 μg/ml Brefeldin A (Sigma) in RPMI 1640 medium supplemented with 10% FBS at 37°C for 24 hours. Following stimulation, the cells were adsorbed with a surface marker, IgG1-FITC MAb (for control), CD4-FITC, or CD-8-FITC MAbs (all Pharmingen). The cells were washed and placed in permeabilization buffer containing 0.125 Vg of rat anti-human IL-10-PE MAb (Pharmingen). A blocked control sample was prepared by adding rat anti-human IL-10-PE MAb with an excess of purified rat anti-human IL-10 MAb (Pharmingen). The cells were incubated and washed as described above.

To measure intercellular IFN-γ, 4×10^6 PBLs were incubated for 6 hours with 20 ng/ml PMA (Sigma), 250 ng/ml ionomycin (Sigma), and 2 μM monensin (Sigma) in RPMI 1640 medium supplement with 10% FBS. Following stimulation, the cells were incubated either with a surface marker, IgG2a-FITC or CD-8-FITC, or CD-3-FITC MAbs (all Pharmingen). After washing, the cells were resuspended in permeabilization buffer containing 0.125 μg of mouse anti-human IFN-γ-PE MAb (Pharmingen). A block control was prepared by adding preincubated mouse anti-human IFN-γPE MAb with an excess of purified mouse anti-human IFN-γ MAb (Pharmingen). The cells were incubated and washed as previously described. All samples were read on a Profile II FACS (Coulter Corporation, Hialeah, FL).

A forward versus side scatter plot was used to set a gate around the lymphocytes. A minimum of 10,000 gated events were acquired per sample. Two-parameter quadrant analyses demonstrating cytokine staining were created using ELITE software (Coulter), and quadrant markers were set using the isotype negative control and the blocked cytokine control to include 99% of the cells in the negative quadrant. Data are presented as percentages of cytokine-expressing cells within the total lymphocyte population gated.

Statistical Analyses

Statistical analyses assessed the effects of stress and age on selected Th-1 and Th-2 cytokines measured in PBLs obtained at two occasions 2 weeks apart. Regression analyses were used to test the effects of two independent variables on each of six cytokine measures. Dependent variables of interest were percent and absolute number of IL-10^+/CD-4^+, IL-10^+/CD-8^+, IFN-γ^+/CD-4^+, IFN-γ^+/CD-8^+ and IL-2^+/CD4^+, and IL-2^+/CD-8^+ cells. For each of these six variables, the mean value of the two separate measures of each variable was used as the dependent variable in the ANOVA. When only a single measure was available, that value was used. The independent variables were (i) age, as a continuous variable, and (ii) a dummy variable coded as 0 to represent control subjects and 1 for caregivers. Regression analyses followed methods recommended by Cohen and Cohen (18) to provide tests of the main effect of each of these predictors as well as their interaction. Results of each test include an F ratio, which provides a significance test, as well as a measure of the variance in the dependent variable accounted for uniquely by the corresponding effect. This approach takes into account the association between group and age mentioned earlier by partialing out each effect from the other.

RESULTS

To determine if our conditions allowed us to accurately determine the percentage of CD-4^+ cells versus IL-10^+ cells, we analyzed the patterns to assess the quality of the data used to calculate the mean values for our analysis. An example of these scatter plots is shown in Figure 1 and is representative of the patterns observed for all the samples.

No significant main effects or interactions were found for analyses of cells expressing IL-2 or IFN-γ. That is, there were no significant effects of stress or age on the percentage or number of IFN-γ^+/CD-8^+, IL-2^+/CD-8^+, IFN-γ^+/CD4^+, or IL-2^+/CD-4^+ cells. However, a consistent pattern of significant effects was obtained for analyses of percentages and absolute numbers of both IL-10^+/CD-4^+ PBLs and IL-10^+/CD-8^+ PBLs. Because the same pattern of results was found for analyses of both percentages and significant numbers of these measures, detailed results are presented for percentages only. In addition, no differences in the mean number of CD-4+ and CD-8+cells between groups before stimulation were found (data not shown).

For percentage of both IL-10^+/CD-4^+ PBLs and IL-10^+/CD-8^+ PBLs, a significant effect of group membership was found: for IL-10^+/CD-4^+ PBLs, $F(1,73) = 5.75$, $p < .05$; for IL-l0^+/CD-8^+ PBLs, $F(1,73) = 13.97$, $p < .001$. For both combinations, the percentage of IL-10+ cells was higher in PBLs obtained from caregivers than in cells from control subjects. Group membership uniquely accounted for 7.30% of the variance in IL-10^+/CD-4^+ PBLs and for 16.10% of the variance in IL-10^+/CD-8^+ PBLs, above and beyond any effect of age. Group means for these two measures are shown in Figures 2 and 3.

For IL-10^+/CD-8^+ PBLs, a significant interaction of stress and age was also obtained, meaning that the influence of age on this measure was significantly different for caregivers and control subjects ($F(1,72) = 6.31$, $p < .05$). The nature of this interaction is shown in Figure 4, which shows a substantial negative influence of age on IL-10^+/CD-8^+ PBLs for caregivers and a rather weak influence for control subjects. From another perspective, the difference between caregivers and control subjects in IL-10^+/CD-8^+ PBLs is reduced considerably with age. The same trend was observed for IL-10^+/CD-4^+ PBLs, although the effect did not reach statistical significance ($F(1,72) = 1.58$, $p = .21$).

Health-Related Behaviors

The majority of older adults take some medication, and the individual who reported no medications was atypical among these subjects. In this sample, 24% used estrogen supplements, 22% took beta blockers or calcium channel

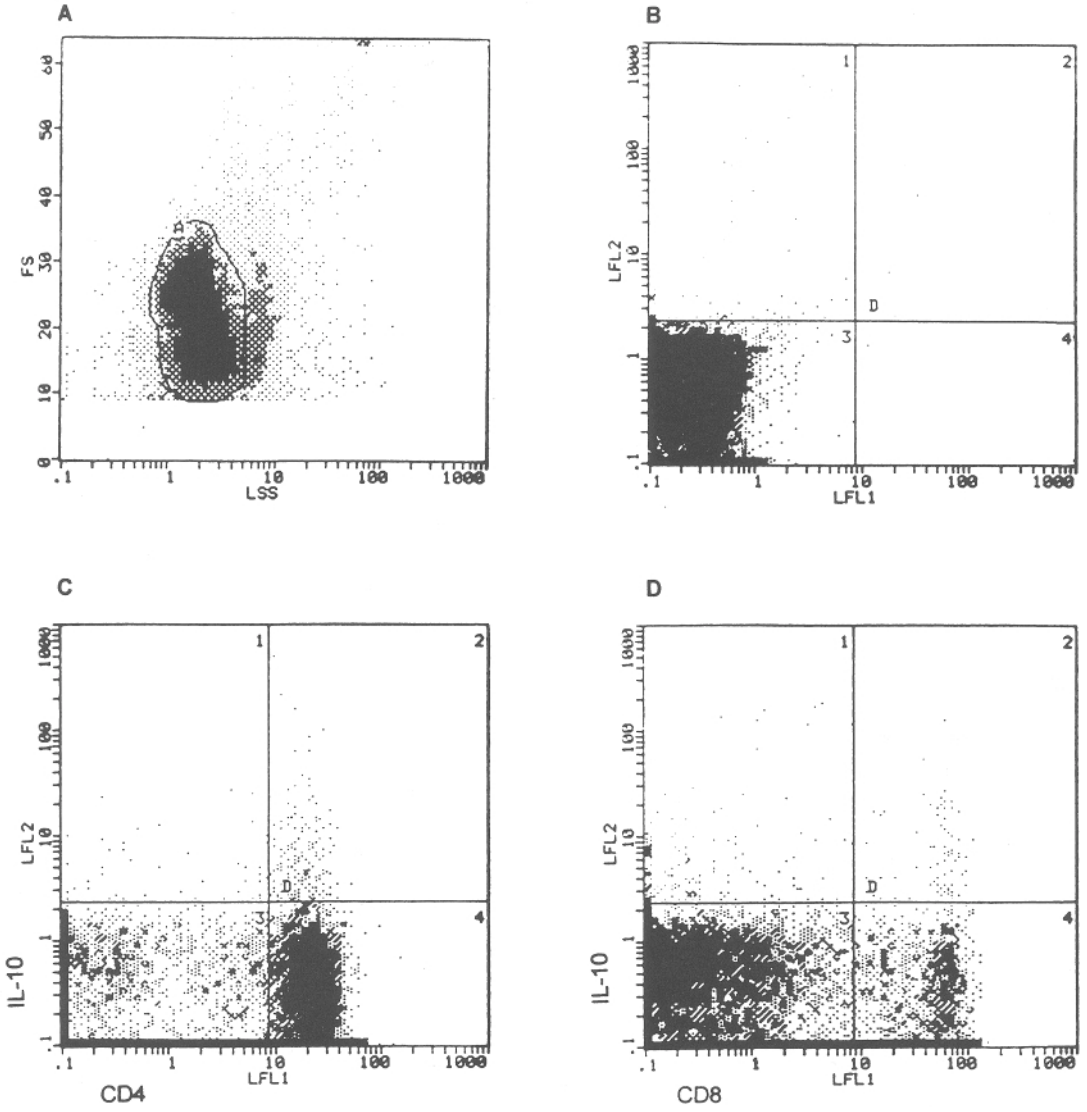

Figure l. Flow cytometric detection of interleukin (IL)-10 in CD-4$^+$ and CD-8$^+$ cells. **A** shows the forward/side scatter distribution and the gate used to select lymphocytes for analysis. **B** shows the quadrant marker set using the isotype and IL-10 blocked controls. **C** and **D** show cells in quadrant 2 that are CD-4$^+$ IL-10$^+$ and CD-8$^+$ IL-10$^+$, respectively.

blockers, 24% used prescription diuretics, and 16% took thyroid supplements, without any systematic group difference. Most other health-related behaviors also failed to distinguish between caregivers and control subjects. Alcohol consumption was low and did not differ between groups; 92% were nonsmokers, without significant group differences. The two groups did not differ in weight change in the prior 2 weeks or hours of sleep within the last 3 days. All subjects had plasma albumin levels (as a measure of nutrition) within the normal range (l9). Caregivers and control subjects did show reliable differences in response to a question about whether they typically engaged in a regular activity long enough to build up a sweat (16), chi square (2, $n = 74$) = 7.24, $p < .05$, with the majority of control subjects and former caregivers answering yes and the majority of current caregivers answering no. However, the variable was not significantly associated with the significant immunologic data. The groups did not differ on any of the health dimensions of the RAND Health Survey (17); all $p > .28$. In sum, there were no reliable differences in health-related behaviors between groups that would have accounted for the immunological differences.

Discussion

In this study we found evidence for a shift from a Th-1, Tcl to a Th-2, Tc2 response associated with caregiving of persons with dementia. Our findings are similar to data reported by Marshall and colleagues (20), who found a similar shift associated with academic stress (an acute stressor) in medical students. We extended their study by measuring the expression of IL-2, IL-10, and IFN-γ in the cytoplasm of CD-4$^+$ and CD-8$^+$ T cells obtained from older individuals

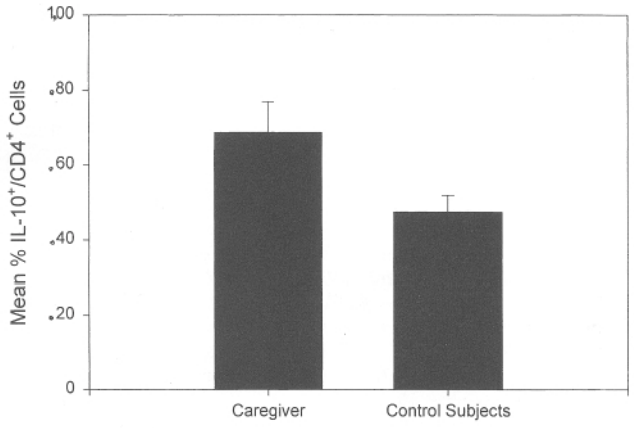

Figure 2. Effect of stress on percent interleukin (IL)-10$^+$/CD4$^+$ peripheral blood leukocytes (PBLs). PBLs were treated with 10 ng/ml lipopolysaccharide and 1 μg/ml Brefeldin in complete RPMI 1640 medium for 24 hours at 37°C. The percentage of CD4$^+$ cells expressing cytoplasmic IL-10 was determined using flow cytometry as described in Methods.

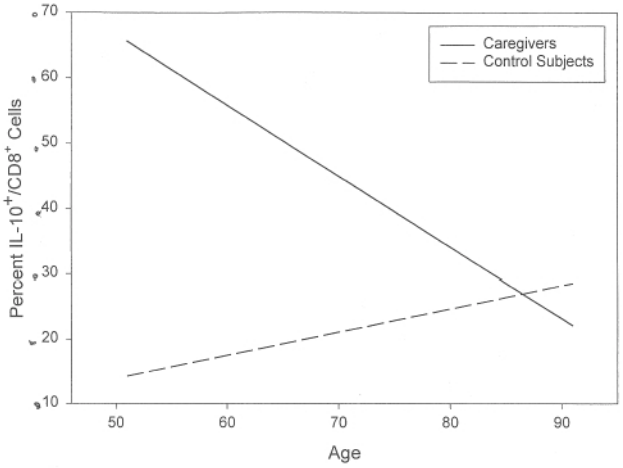

Figure 4. Interactive effect of stress and age on percent interleukin (IL)-10$^+$/CD8$^+$ cells. A significant interaction of stress and age was found between caregivers and control subjects.

caregiving for a patient with dementia, a chronic stressor, using flow cytometry. Although caregiving was not associated with the percentage or total number of either CD-4$^+$ or CD-8$^+$ cells expressing IL-2 or IFN-γ, there was a significant increase in the percentage and total number of IL-10$^+$/ CD-4$^+$ and IL-10$^+$/CD-8$^+$ cells. Additionally, this pattern was significantly stronger in younger individuals when compared with older subjects, indicating that there may be an aging component to the Th-1, Tcl to Th-2, Tc2 shift. Whereas data from mouse studies suggest that a Th-1 to Th-2 shift is associated with aging, data from human studies are less well defined (21). The range of the percentages of IL-10$^+$ cells found in this study is consistent with normal values of IL-10$^+$ PBLs (Podma Kodukula, Pharmingen, personal communication, April 2000) as well as previously published data (22).

The absence of immunologic differences between current and former caregivers is consistent with prior work (4,10)

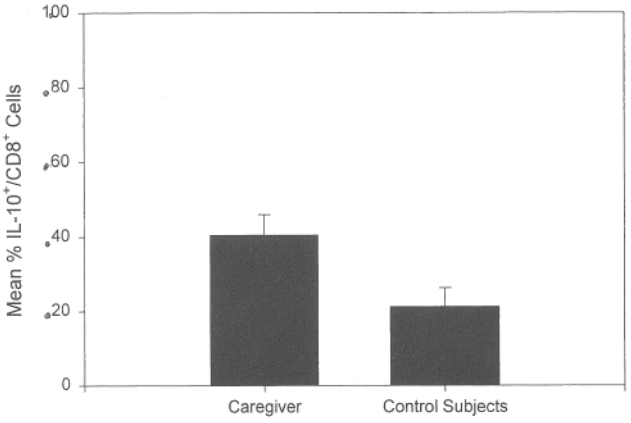

Figure 3. Effect of stress on percent interleukin (IL)-10$^+$/CD8$^+$ peripheral blood leukocytes (PBLs). PBLs were treated as described for Figure 1. The percentage of CD8$^+$ cells expressing cytoplasmic IL-10 was determined by flow cytometry as described in Methods.

and suggests that physiological and psychological consequences of chronic stressors may persist well beyond the cessation of the actual stressor. Indeed, research with other populations suggests that such effects are not unique to caregivers; other data suggest that continued immunologic down-regulation may be related to persistent rumination about a past stressful event (23), consistent with rumination data from former caregivers (24). However, because of age-related immunological changes, older adults may be the most vulnerable to actual health changes.

In previous work using an acute academic stress model with medical students, we found a significant decrease in the production of IFN-γ by PBLs obtained at the time of examinations as compared with PBLs obtained at baseline (25). These data were confirmed by Marshall and colleagues (20). In an earlier study, we did not find a difference in the production of IFN-γ by PBLs stimulated in vitro in caregivers versus control subjects (unpublished data). In this study we confirmed these results and found that the percentage and total number of CD-4$^+$ or CD-8$^+$ lymphocytes expressing IFN-γ were the same for the Alzheimer's disease caregivers and control subjects. We do not know if the differences in the results with regard to IFN-γ and in the medical student studies versus the study with caregivers are related to age, the effects of a chronic stressor versus an acute stressor, or a combination of both. However, consistent with the study by Marshall and colleagues (20), we did find an increase in the number of CD-4$^+$ and CD-8$^+$ cells expressing cytoplasmic IL-10. As already discussed, IL-10 will down-regulate IFN-γ production and will tend to dampen the overall cellular immune response (12).

One possible explanation for a shift from Th-1, Tel to Th-2, Tc2 lymphocytes may be related to changes in catecholamine levels, which are elevated as a result of psychological stress. Elenkov and Chrousos (26) recently reported that an increase in IL-10 production was associated with an increase in catecholamines, and these changes resulted in a shift in the Th-l to Th-2 direction. Possible implications for hypothalamic pituitary adrenal axis activation and a shift

from Th-1 to Th-2 cytokine responses has also been demonstrated (27). Previous data from our laboratory and others on the impact of dementia caregiving on the cellular immune response and the data obtained in this study are consistent with these reports and support the hypothesis that stress-associated hormones can induce cytokine dysregulation.

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REFERENCES

1. Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R. Spousal caregivers of dementia victims: longitudinal changes in immunity and health. *Psychosom Med.* 1991;53:345-362.
2. McCann JJ. *Effects of stress on spouse caregivers' psychological health and cellular immunity* (dissertation). Chicago, IL: Rush University College of Nursing; 1991.
3. Irwin M, Smith TL, Christian G. Electroencephalographic sleep and natural killer activity in depressed patients and control subjects. Psy*chosom Med.* 1992;54:10-21.
4. Esterling BA, Kiecolt-Glaser JK, Glaser R. Psychosocial modulation of cytokine-induced natural killer cell activity in older adults. *Psychosom Med.* 1996;58:264-272.
5. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. *Lancet.* 1995; 346:1194-1196.
6. Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB, Sheridan J. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc Natl Acad Sci USA.* 1996;93:3043-3047.
7. Gravenstein S, Drinka P, Duthie EH, et al. Efficacy of an influenza hemagglutinin-diphtheria toxoid conjugate vaccine in elderly nursing home subjects during an influenza outbreak. *J Am Geriatr Soc.* 1994; 42:245-251.
8. Vedhara K, Cox NKM, Wilcock GK, et al. Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *Lancet.* 1999;353:627-631.
9. Glaser R, Rabin B, Chesney M, Cohen S, Natelson B. Stress-induced immunomodulation: implications for infection diseases? *JAMA.* 1999; 281:2268-2270.
10. Glaser R, Kiecolt-Glaser JK, Malarkey WB, Sheridan JF. The influence of psychological stress on the immune response to vaccines. *Ann NYAcad Sci.* 1998;840:656-663.
11. Wu H, Wang J, Cacioppo JT, Glaser R, Kiecolt-Glaser JK, Malarkey WB. Chronic stress associated with spousal caregiving of patients with Alzheimer's dementia is associated with down-regulation of B-lymphocyte GH mRNA..1 *Gerontol Med Sci.* 1999;54:M212-M215.
12. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today.* 1996;17:138-146.
13. Elenkov 1, Papanicolaou D, Wilder R, Chrousos G. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *Proc Assoc Am Physicians.* 1996;108:374-381.
14. Dobbs CM, Feng N, Beck FM, Sheridan JF. Neuroendocrine regulation of cytokine production during experimental influenza viral infection: effects of restraint stress-induced elevation in endogenous corticosterone. *J Immunol.* 1996;157:1870-1877.
15. Kiecolt-Glaser JK, Glaser R. Methodological issues in behavioral immunology research with humans. *Brain Behav Immun.* 1988;2:67-78. 16. Washburn RA, Adams LL, Hale GT. Physical activity assessment for epidemiologic research: the utility of two simplified approaches. *Prev Med.* 1987;16:626-646.
17. Ware JE, Sherbourne CD. The MOS 36-Item-Short-Form Health Survey (SF-36): 1. Conceptual framework and item selection. *Med Care.* 1992;30:473-483.
18. Cohen J, Cohen *P. Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences.* 2nd ed. Hillsdale, NJ: Erlbaum; 1983.
19. Cooper JK, Gardner C. Effect of aging on serum albumin. *J Am Geriatr Soc.* 1989;37:1039-1042.
20. Marshall GD Jr, Agarwal SK, Lloyd C, Cohen L, Henninger EM, Morris GJ. Cytokine dysregulation associated with exam stress in healthy medical students. *Brain Behav Immun.* 1998;12:297-307.
21. Karanfilov CI, Liu B, Fox CC, Lakshmana R, Whisler RL. Age-related defects in Thl and Th2 cytokine production by human T cells can be dissociated from altered frequencies of CD45RAI and CD45ROI T cell subsets. *Mech Ageing Devel.* 1999;109:97-112.
22. Crucian B, Dunne P, Friedman H, Ragsdale R, Pross S, Widen R. Detection of altered T Helper 1 and T Helper 2 cytokine production by peripheral blood mononuclear cells in patients with multiple sclerosis utilizing intracellular cytokine detection by flow cytometry and surface marker analysis. *Clin Diagn Lab biannual.* 1996;3:411-416.
23. Solomon GF, Segerstrom SC, Grohr P, Kemeny M, Fahey J. Shaking up immunity: psychological and immunological changes after a natural disaster. *Psychosom Med.* 1997;59:114-127.
24. Bodnar J, Kiecolt-Glaser JK. Caregiver depression after bereavement: chronic stress isn't over when it's over. *Psychol Aging.* 1994;9:372-380.
25. Glaser R, Rice J, Speicher CE, Stout JC, Kiecolt-Glaser JK. Stress depresses interferon production by leukocytes concomitant with a decrease in natural killer cell activity. *Behav Neurosci.* 1986;100:675-678.
26. Elenkov IJ, Chrousos GP. Stress hormones, Thl/Th2-patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends Endocrinol Metab.1999;10:359-368.*
27. Agarwal SK, Marshall GD Jr. Glucocorticoid induced type-1/type-2 cytokine alterations in humans: a model for stress-related immune dysfunction. *J Interferon Cytokine Res. 1998;18:1059-1068.*

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