The Influence of Psychological Stress on the Immune Response to Vaccines[a]

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ABSTRACT: We compared virus-specific antibody and T-cell responses to influenza virus vaccination in 32 caregivers of Alzheimer's disease (AD) patients and matched control subjects. Caregivers showed a poorer antibody response and virus-specific T-cell response following vaccination compared to the control subjects as measured by fourfold increases in antibody titers to the vaccine and lower levels of virus-induced IL-2 levels *in vitro*. We performed a second study in which forty-eight medical students were inoculated with a series of three injections of the hepatitis-B (HEP-B) vaccine to coincide with the third day of three, three-day examination blocks. Twelve of the 48 medical students seroconverted after the first injection; these students were characterized by falling into the lower stressed/lower anxiety group of students. Students who reported greater social support and lower anxiety and stress demonstrated a higher antibody response to the vaccine and a more vigorous T-cell response to HEP-B surface antigen at the end of the third examination experience. The differences in antibody and T-cell responses to HEP-B and influenza virus vaccinations provide a demonstration of how stress may be able to alter both the cellular and humoral immune responses to vaccines and novel pathogens in both younger and older adults.

The stress of caring for a relative with a progressive dementia such as Alzheimer's disease has multiple adverse effects on caregivers.[1,2] In studies from our laboratory and others, caregivers were more distressed and had higher rates of syndromal depressive and anxiety disorders than category-matched (age, sex, and SES) comparison subjects who did not have any caregiving responsibilities.[1-8]

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Caregivers also had poorer immune function, more respiratory tract infections, a poorer antibody and virus-specific T-cell response to influenza virus vaccine, and slower wound healing than noncaregivers.[9–16]

In work from other laboratories, depression in spousal caregivers has been linked to impaired proliferative responses and declines in lymphocytes with surface signal transduction molecules.[17] Irwin *et al.*[18] reported that plasma levels of neuropeptide Y (NPY), a sympathetic neurotransmitter, were elevated in spousal caregivers compared to controls and NPY was negatively correlated with natural killer (NK) cell cytotoxicity among caregivers. Delayed hypersensitivity skin testing was markedly poorer in 34 spousal caregivers than 33 noncaregivers[19]; in fact, compared to normal age and gender standards, 50% of the caregivers were totally or relatively anergic, compared to only 12% of noncaregivers.

Importantly, preliminary data support that cessation of caregiving does not terminate risk; in the data collected thus far in our longitudinal study, bereaved (i.e., former) spousal caregivers have not differed significantly from continuous caregivers on cellular immune function or depression after the death of the impaired spouse. In fact, spousal caregivers have continued to show immunological downregulation as well as increased risk for syndromal depressive disorders for several years after bereavement.[14,15,20]

For example, NK cells from caregivers showed a decrease in the inability to respond to the stimulatory effects of recombinant interferon-γ (rIFN-γ) and recombinant interleukin-2 (rIL-2) than NK cells from controls.[14] Current and former caregivers did not differ from each other, and both showed poorer responses to these cytokines than controls. When caregivers were divided into 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 cytokine), low responders reported less positive emotional and tangible supports, and rated less closeness in their relationships, compared to the high responders.[14] High and low responders did not differ on depressive symptoms or perceived stress.

A follow-up study using cell preparations enriched for NK cells (approximately 90%) replicated the previously observed group differences in the NK cell lysis response to rIFN-γ and rIL-2.[15] In addition, higher levels of social support were associated with heightened NK cell responses to cytokines, independent of level of depression.

The immunological downregulation associated with caregiving is noteworthy because aged individuals already have diminished immune function involving T-cells and cytokine levels, and these age-related reductions have important health consequences: influenza and pneumonia are responsible for high rates of morbidity and mortality among older adults.[21] Thus, it is of particular concern that current caregivers and controls also differ significantly in both their T-cell and antibody responses to influenza vaccination.[13] Adults who show poorer responses to vaccines and other antigenic challenges also experience higher rates of clinical illness, including influenza virus infections.[22–24] These data suggest that caregivers are more vulnerable than their age-peers to influenza virus infection and, potentially, to other infectious agents.[22–24]

To further address the question of persistent immunological deficits in former caregivers, we collected data across two consecutive flu seasons. The 124 subjects who were inoculated in the first season included 75 controls (mean age = 70.67, SEM = 1.83), 23 current spousal caregivers (mean age = 71.92, SEM = 1.72), and 26 former caregivers (mean age = 72.65, SEM = 1.70); for the latter, an average of 23.84 months (SEM = 3.3) had elapsed since the death of their impaired spouse.

Subjects in the longitudinal study had been asked annually if they had received a flu shot. Caregivers reported an average of 1.62 (SEM = 0.19) inoculations over the last three years, compared to 1.21 (SEM = 0.15) in controls, $F(1,120) = 2.62$, $p < 0.10$. Accordingly, caregivers might have been expected to show somewhat better baseline vaccine responses before the current inoculation, for two reasons: the "B" component of the trivalent vaccine had not changed over the prior two years, and there is some cross-reactivity among influenza subtypes (and thus among vaccine components).

Despite caregivers' relative advantage over controls with respect to their recent vaccination history, caregivers had significantly *lower* antibody titers than controls to the vaccine (as measured by ELISA, with Fluzone® vaccine used as the antigen), $F(1,118) = 8.83$, $p < 0.004$; moreover, bereaved and continuing caregivers did not differ, $F = 0.01$. Residualized gain scores were computed to control for baseline differences. In addition to the obvious differences at baseline between groups, caregivers showed less of an increment in antibody two weeks after vaccination as measured by ELISA. Thus, caregivers had lower antibody titers at baseline than control subjects, and lower antibody titers in response to vaccination.

In addition to the differences in antibody titers, the persistence of the T-cell response (as measured by the ability of PBLs to synthesize IL-2 after stimulation with Fluzone vaccine) also differed significantly between caregivers and controls three months post inoculation (FIG. 1). In summary, this initial study showed that caregivers had both a poorer antibody response and a more rapid decline in the virus-specific T-cell response over time than control subjects.

For the second vaccine season, subjects were only included in these analyses if they had received an influenza virus vaccination in the prior year. Thus, the groups had comparable vaccine histories. The sample included 68 controls (mean

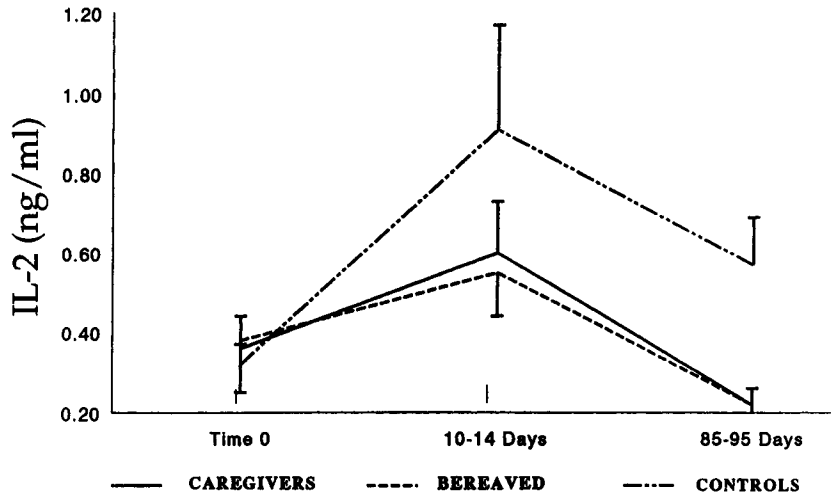

FIGURE 1. ***In vitro*** **IL-2 responses to Fluzone vaccine** (mean ± SEM) at the time of vaccination and two weeks and three months after vaccination for those subjects who showed at least a fourfold antibody increase. These data represent a specific T-cell response to the vaccine.

age = 71.54, SEM = 1.05), 32 current spousal caregivers (mean age = 73.12, SEM = 1.53), and 22 former caregivers (mean age = 72.77, SEM = 1.82). The 22 former caregivers had been bereaved an average of 29 months (SEM = 3.81), with a range from two months to five years. Importantly, in this sample, where all subjects had the same recent vaccine histories, comparisons of prevaccination antibody titers as measured by ELISA showed no differences between current caregivers, former caregivers, and noncaregivers.

Although caregivers and noncaregivers had comparable baseline antibody titers as measured by ELISA, caregivers (both current and bereaved) responded less often than controls after vaccination, as shown in FIGURE 2. Among the 22 former caregivers, only 7 (32%), showed a fourfold or greater response to any one of the three individual vaccine components, or to the total Fluzone vaccine when used as the antigen (i.e., all three hemagglutinin components combined), compared to 12/32 (38%) of current caregivers and 40/68 (59%) of controls. Although current and former caregivers did not differ from each other in the proportion who showed a fourfold increase in antibody titers, both differed significantly from control subjects, $\chi^2(2) = 6.42$, $p < 0.04$.

Because only those subjects who had shown a fourfold increase in antibody were followed through the three and six-month time points, virus-specific T-cell response data (IL-2 levels) were limited to the 7 of the 22 subjects who had shown an antibody response. However, former caregivers showed an equivalent or poorer antibody and T-cell response than current caregivers within this small sample (see IL-2 data in Kiecolt-Glaser *et al.*).[13] Thus, consistent with prior data, bereaved caregivers' responses to influenza virus vaccination continued to lag behind those of controls while not differing from current caregivers.

Most health-related behaviors did not distinguish between caregivers and controls. Alcohol consumption was low and did not differ between groups. The two groups did not differ in body mass or weight change in the prior week. All subjects had plasma albumin levels (as a measure of nutrition) within normal range.[25]

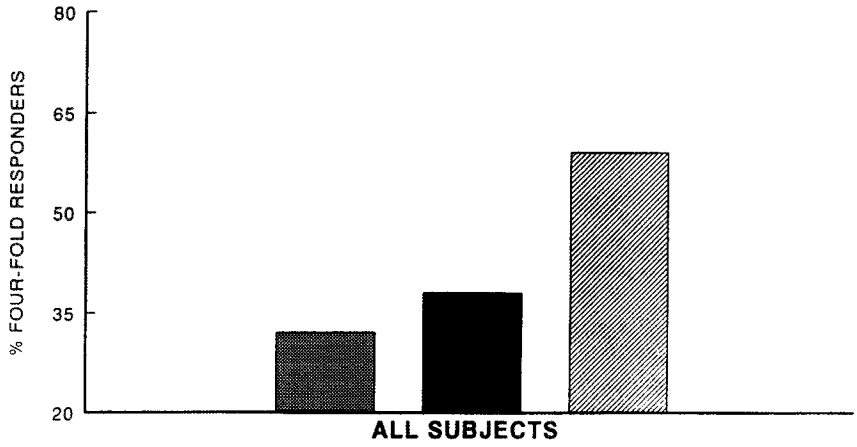

FIGURE 2. Percentage of current and former caregivers and controls showing a clinically significant fourfold increase in antibody one month after vaccination using ELISA antibody data. Former caregivers; current caregivers; controls.

Caregivers and controls did show reliable differences in sleep and exercise, but neither variable was significantly correlated with immunological data. In sum, there were no reliable differences in health-related behaviors between groups that would have accounted for these immunological differences.

Thus, in two separate studies caregivers showed clear deficits relative to controls in both their cellular and humoral immune responses to influenza virus vaccination: caregivers were less likely to show a significant increase in antibody titers four weeks after vaccination as measured by two independent procedures, ELISA and hemagglutination inhibition. When PBLs were stimulated with lipopolysaccharide (LPS) to stimulate monocytes to produce IL-1β, a cytokine important for an antibody response, PBLs from caregivers produced lower levels of IL-1β. The protective capacity of vaccines is dependent on their ability to induce both humoral and cell-mediated immune responses[26-29]; both were poorer in caregivers than controls, and current caregivers did not differ from former caregivers.

The NK cell lysis data from our earlier two studies[14,15] are consistent with results from the influenza vaccine studies, with controls showing a significantly better response than either bereaved or continuing caregivers, while the two caregiver groups do not differ from each other. Thus, evidence from several studies indicate that bereaved caregivers demonstrate persistent immunological downregulation, consistent with their continued elevated risk for syndromal depression.[20]

Other studies have shown stress-related modulation of vaccine responses in young and healthy subjects. "Academic" stress was related to alterations in both the cellular and humoral immune response to a recombinant hepatitis B virus vaccine in medical students,[30] a finding replicated in another study.[31] In addition, Cohen *et al.*[32] showed that stress altered susceptibility to several different strains of respiratory viruses in a controlled laboratory study where subjects were inoculated with the same dose of virus. However stress-related immunological alterations are likely to have their most potent health consequences in older adults and other at-risk populations who already have impairments in immune function: Older adults show greater immunological impairment related to stress or depression than younger individuals.[33] Age-related immunological declines are related to the increased vulnerability to infectious illness among the elderly.[21-29]

Other work has addressed relationships between chronic stress and growth hormone (GH) gene expression in peripheral blood mononuclear cells (PBMCs). Malarkey *et al.*[16] found differences in GH mRNA levels in PBMCs of spousal caregivers compared to age- and weight-matched control groups; in fact, there was no overlap in GH mRNA levels between the control and caregiver populations. Therefore, the chronic stress documented in caregivers appears to be associated with a decrease in GH gene expression in their PBMCs. Because GH is an immune-enhancing hormone, it is possible that these data provide a clue to the mechanism(s) whereby psychological stress may downregulate the immune response. These results also support the hypothesis that experiences associated with caregiving can alter the autonomic nervous system and neuroendocrine control of the HPA axis. Thus, the decrease in GH mRNA found in PBMCs of caregivers may be partially responsible for the poor immune response to influenza virus vaccination and delayed wound healing observed in our studies.[12,13]

These data suggest that physiological and psychological consequences of chronic stressors may persist well beyond the cessation of the actual stressor. Indeed, research with other populations suggests such effects are not unique to caregivers; other data suggest that continued immunological downregulation may be related to persistent rumination about a past stressful event[34,35] consistent with rumination

data from bereaved caregivers.[20] However, because of age-related immunological changes, older adults may be most vulnerable to actual health changes.

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