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Caregiving is Associated with Low Secretion Rates of Immunoglobulin A in Saliva.

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Abstract

Although the chronic stress of caring for a sick/disabled relative has been associated with poorer immunity using a range of outcomes, its impact on secretory immunoglobulin A (S-IgA) in saliva has yet to be examined. Three hypotheses were tested in analyses of data from a large community sample: first, caregivers would have lower S-IgA secretion rates than non-caregivers; second, the impact of caregiving on S-IgA would be particularly apparent in older participants; third, for caregivers, caregiving burden would be negatively associated with S-IgA. The sample comprised three distinct age cohorts, one young (N = 623), one middle aged (N = 639), and the other elderly (N = 582). Participants were classified as caregivers if they regularly cared for somebody other than routine childcare. Caregiving strain was measured and a caregiving burden index was then derived as the composite of the number of people being cared for, the type of care provided, and the residential status of the person being cared for. From 2-min saliva samples, S-IgA secretion rate was measured. There was a significant caregiver status by age cohort interaction; caregivers in the eldest cohort had lower S-IgA secretion rates than their non-caregiving counterparts. Caregiving strain and burden and S-IgA were related, such that caregivers who experienced greater strain and burden had lower S-IgA secretion rates. These findings resonate with those from other studies using different immune outcomes. Considered together, it is clear that that the chronic stress of caregiving has widespread effects on immunity.

Keywords: Age; Caregiving; Chronic stress; Secretory Immunoglobulin A

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1. Introduction

In humans, caregiving for a sick or disabled relative has frequently been employed as a model for examining the effects of chronic stress on immune function (Baron et al., 1990; Cohen and Pollack, 2005; Gennaro et al., 1997; Reese et al., 1994; Vedhara et al., 1999; Vedhara et al., 2002; Vitaliano et al., 1998). Studies have assessed the impact of such caregiving on the antibody response to vaccination, rate of wound healing, and aspects of cellular immunity. For example, in comparison to matched controls, caregivers mounted a poorer response to both influenza and pneumococcal vaccinations (Glaser et al., 2000; Kiecolt-Glaser et al., 1996; Vedhara et al., 1999), had reduced natural killer cell cytotoxicity (Cohen and Pollack, 2005), took on average nine days longer to heal from punch biopsy wounds (Kiecolt-Glaser et al., 1995), and displayed poor control of latent viruses (Kiecolt-Glaser et al., 1987). Given the consensus that immune down-regulation is associated with caregiving, it is perhaps surprising that secretory immunoglobulin A (S-IgA) has not been studied in this context. S-IgA constitutes the primary defence against mucosal infections and the vast majority of infections occur at the mucosal level (Bosch et al., 2002; Brandtzaeg, 2003). In addition, caregivers have a higher incidence of oral and upper respiratory tract infections (Dyck et al., 1999; Vitaliano et al., 2005), and take longer to recover from such illnesses (Kiecolt-Glaser et al., 1991) than controls.

The evidence linking prolonged or ongoing stress to S-IgA has tended to concentrate on more general life events stress, with lower secretion rates being associated with fewer desirable life events, (Evans et al., 1993) and more undesirable events (Evans et al., 1994; Ng et al., 2004; Stone et al., 1994). Recently, we reported that the stress load imposed by major life events was negatively associated with S-IgA secretion rate in a large community sample, even after adjustment for a number of confounders including smoking, occupation status, and saliva volume (Phillips et al., 2006). It should be acknowledged, however, that life events stress may be a less than perfect way of conceptualising chronic stress, particularly for certain populations. Despite having an undesirable effect, a life event may be secondary in terms of health impact relative to the continuing toll taken by the negative circumstances in which that particular event is embedded (Pearlin, 1989). Problems that are enduring, persistent, and threatening on a daily basis, that are often tied to social roles, have been regarded as affording a better model of chronic

stress (Pearlin, 1989). This makes it doubly curious that the impact of caregiving on S-IgA has yet to be studied.

When carers are continuously providing care on a daily basis, they frequently struggle financially, physically, psychologically and socially (Majerovitz, 2007; Pinquart and Sorensen, 2003b), and usually it is along these dimensions where they differ from non-caregivers (Baron et al., 1990; Kiecolt-Glaser et al., 1987; Kiecolt-Glaser et al., 1995; Pariante et al., 1997). The term 'caregiver burden' has been coined to describe the overall impact of the stress of caregiving on these dimensions (for review see Pinquart and Sorensen, 2003a, b). Burden appears to be fairly stable over time (Vedhara et al., 2000), implying a consistency in chronic stress exposure. Family caregivers who experience greater caregiving burden have been found to report more physical and psychological health problems (Forbes et al., 2007; Pinquart and Sorensen, 2003b). In addition, in parents caring for a child with a disability, higher caregiver burden was negatively associated with T helper/suppressor cell ratio, suggesting a poorer ability to regulate cellular immunity (Pariante et al., 1997).

The present study examined the effects of chronic caregiving stress and burden on S-IgA secretion rates using data from a large adult community sample. It was hypothesized that caregivers would have lower S-IgA secretion rates than non-caregivers. In contrast to the outcomes from vaccine studies in older spousal caregivers of dementia patients (Esterling et al., 1996; Glaser et al., 2000; Vedhara et al., 1999), no difference in antibody response to the influenza vaccination was observed between younger spousal caregivers of multiple sclerosis patients and matched controls (Vedhara et al., 2002). Although other explanations for this discrepancy in results can be entertained, it is possible that caregiving has its greatest impact in those who through immunosenescence already have less than optimal immune systems (Graham et al., 2006). Accordingly, it was also hypothesized that caregiver effects on S-IgA would be particularly evident in older participants. Given the unique composition of this sample, with three distinct age cohorts, we were able to examine this proposition. Finally, it was hypothesized that, for caregivers, caregiving strain and burden would be negatively associated with S-IgA; those with the highest strain and burden would exhibit the lowest S-IgA secretion rates.

2. Method

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2.1. Participants

Data were derived from the West of Scotland Twenty-07 Study. Individuals were all from the Glasgow area and have been followed up at regular intervals since the baseline survey in 1987 (Ford et al., 1994). Members of the youngest cohort were all aged around 24 years old, the middle cohort were all around 44 years old, and members of the older cohort were all around 63 years old at the third follow-up when data on caregiving were collected and a saliva sample taken. The effective sample size was 1843, comprising 623 from the youngest, 638 from the middle, and 582 from the eldest cohort. Overall, there were 846 (46%) men and 997 (54%) women. Of the 98% who gave socio-economic status information, 878 (48%) of the participants came from manual and 934 (52%) from non-manual occupational households. The sex ratios for the three cohorts were virtually identical: 292 (47%) men and 331 (53%) women for the younger cohort; 282 (44%) men and 356 (56%) women for the middle cohort; and 272 (47%) men and 310 (53%) women for the older cohort. However, the distribution of participants from manual and non-manual occupational households differed significantly between cohorts (χ^2 (2) = 26.0, p < 001); there were more participants from manual than non-manual occupational households in the older cohort, than in the other two cohorts. In the younger and middle cohorts, 272 (45%) and 275 (44%) came from manual occupational households, respectively, whereas in the older cohort, 331 (57%) came from manual occupational households. This reflects secular changes in the occupational structure in the UK (Der, 1998).

2.2. Apparatus and procedure

Participants were tested in a quiet room in their own homes by trained nurses. Demographic information was obtained by interview. Household socioeconomic position was classified as manual and non-manual from the current or previous occupational status of the head of household, using the Registrar General's (1980) Classification of Occupations. Head of household was the man, unless the man had not been employed; in such circumstances, the

women's current/previous occupation was used. For the younger cohort, the main breadwinner of the household was the head of household.

Information about caregiving was ascertained firstly by asking participants "Is there anyone who regularly depends on you for help or support? By that I mean that you do things they couldn't manage for themselves because of illness, disability, old age, or some other reason?" This was not to include any routine childcare. If participants answered "yes" to this question, they were then invited to list up to four people to whom they gave this help. For each of the named persons, they were asked: what did they help them with; how many hours did they spend helping in an average week; how much of a strain is it (answered on a scale ranging from 1 = no strain at all, to 5 = a great strain); and does this person live in the same household as you (resident in the same household; lives in another household; lives in an institution). Types of help given included: personal care, mobility round house, household chores, transportation outside, dealing with officials, financial affairs etc., companionship, or everything. This was coded such that each type of help was assigned a value of 1, and summed to a maximum value of 7; if participants said they helped with everything, this was coded as 7.

Three caregiving measures were then derived from this information: total hours per week caregiving, a strain score, and a total caregiving burden score. The hours per week spent caregiving for each person the participant cared for was summed to produce a total hours per week caregiving variable. Strain was also summed across the number of people the participant was caregiving for to give the strain score. The types of help variables were summed across the number of people the participant was caregiving for, and a composite residence score was calculated by coding residence in the same household as 3, living in another household as 2, and living in an institution was as 1, and summing across the number of people the participant was caregiving for, such that a higher score indicated that participants were more likely to be living with the person(s) needing care. For types of help, it is difficult to judge which activities are more burdensome (e.g. helping with transportation versus household chores); therefore, each was weighted identically and burden judged to be greater if more types of help were being provided. Since the stress of caring for a family member with Alzheimer disease is associated with the number of instrumental behaviours that the care recipient requires help with (Gonzalez-Salvador et al., 1999), this would seem a reasonable way of proceeding. With regard to the residence

score, we assumed that the burden would be greatest if the person being cared for was co-resident and least if the person was living in an institution. Since caregivers of spouses with dementia who were co-resident have been reported to show poorer natural killer cell profiles than those with institutionalized spouses, this would seem a reasonable assumption (Kiecolt-Glaser et al., 1987). It was further assumed that total burden would reflect the demands that come with, for example, co-residency, the aggregate types of help required by the care recipient, and the number of people to whom care was provided. Thus, a total caregiving burden score was derived as the product of the total for types of help and composite residence score.

Smoking status was determined by responses to the question, 'Do you ever smoke tobacco now? I am thinking of a pipe, cigars and your own roll ups as well as cigarettes you might buy.' If the answer was 'No', participants were asked, 'Did you ever used to smoke any kind of tobacco?' On this basis, participants were characterised as 'never smokers', ex smokers', or 'current smokers'.

Saliva samples were taken at the end of the interview, using standard salivettes (Sarstedt Ltd, Leicester, UK). Participants were instructed to swallow hard to dry out the mouth and then immediately to place the swab under the tongue. They were asked to hold the swab as still as possible for 2 minutes, timed precisely by the nurse interviewers using portable digital timers. After exactly 2 minutes, participants removed the swab, returning it to the salivette case. It should be conceded that the use of absorbent cotton materials to collect saliva has been observed to produce lower S-IgA concentrations (Shirtcliff et al., 2001); however, it is reasonable to consider that any such reduction would be a constant error. All samples were frozen within 2 hours of collection and remained frozen at -20 degrees C until assay. Samples were dispatched from Glasgow to London by air for assay in four batches and recovered after thawing by centrifugation at 1000g for 10 minutes. Double antibody sandwich ELISA, described in detail elsewhere (Carroll et al., 1996), was used to determine S-IgA concentration. Intra-assay % coefficient of variation was 3.8 and inter-assay % coefficient of variation was 7.6. Prior to assay, saliva volume was determined gravimetrically. Saliva sample collection and volume determination for S-IgA analysis conformed to a procedure described previously (Zeier et al., 1996). The focus of analysis was S-IgA secretion rates (μg/2min), which were calculated as the product of saliva volume (ml) and S-IgA concentration (μg/ml).

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2.3. Data analyses

Analysis was undertaken using SPSS version 15. The main derived caregiving variables (time spent caregiving per week, caregiving strain, and total burden score) were positively skewed, so were subject to $\log_{10}$ transformations prior to analysis which reduced the skewness. Means and standard deviations, however, are presented for the untransformed data for descriptive purposes. S-IgA secretion rate values were highly variable and this was reduced by $\log_{10}$ transformation; we have adopted this strategy in numerous other studies (Phillips et al., 2006; Ring et al., 2002). ANOVA was used to compare all caregiving scores between cohorts, sexes, and household occupational groups. Eta-squared (η^2) was used as an indicator of effect size. Similar analyses were applied to the $\log_{10}$ transformed S-IgA secretion rate values. ANCOVA was then applied to determine whether caregivers and non caregivers differed on S-IgA secretion rate, with assay batch, saliva volume, and smoking behavior as covariates. Since the batch variable reflected inter-assay variation, it could be a potential confounder. Adjustment for saliva volume would help establish whether any associations between S-IgA secretion rates and caregiving were driven by variations in saliva volume or reflected a link with actual variations in S-IgA production and/or transport (Willemsen et al., 1998). The importance of controlling for smoking behaviour when examining possible relationships between psychosocial variables and S-IgA has already been demonstrated (Evans et al., 2000). Age cohort was also entered as a fixed factor in these analyses given our *a priori* hypothesis regarding the interaction between age and caregiving.

Analyses were then undertaken within the caregiving group. Hierarchical linear regression was conducted, with $\log_{10}$ transformed S-IgA secretion rate as the dependent variable. In these analyses, assay batch, saliva volume, and smoking behavior were entered at step 1, and the main derived caregiving variables (time spent caregiving per week, caregiving strain, total burden score) were entered separately at step 2. In subsequent analyses, batch, smoking, and saliva volume were again entered at step 1, with age cohort and each caregiving variable in turn entered at step 2, and the interaction between age cohort and the caregiving variable at step 3. In order to generate the interaction terms, and as recommended to avoid multicolinearity (Aiken and

West, 1991; West et al., 1996), the caregiving variables were mean centred and the product of these variables and age cohort derived. For these regressions, β, the standardized regression coefficient, was reported as a measure of association, t as a means of testing significance, and the change in R-squared as the measure of effect size.

3. Results

3.1. Caregiving and demographic variables

Three hundred and thirty four participants answered yes to caregiving for someone other than their own or others' children. There were significantly more caregivers in the middle and elder cohorts, $\chi^2(2) = 56.44$, $p < .001$, than in the younger cohort, with 56 (17%) in the younger cohort, 158 (47%) in the middle, and 120 (36%) in the older cohort. There were significantly more female caregivers (200, 60%) than male caregivers (134, 40%), $\chi^2(1) = 5.50$, $p = .02$; in addition, there was a small but statistically significant, $\chi^2(1) = 5.14$, $p = .02$, excess of caregivers from manual occupational households (177, 54%) compared to caregivers from non-manual occupational households (150, 46%).

For caregivers, there were no differences between the cohorts in terms of the types of help provided, the total residency score, or the ratings of caregiving burden. However, there was a positive linear relationship between age and hours per week spent caregiving, $F(2,320) = 5.46$, $p = .005$, $\eta^2 = .033$; the older cohort spent most time caregiving and the younger cohort the least number of hours. There was also a trend for the older two cohorts to report greater caregiving strain than the younger cohort, $F(2,327) = 2.68$, $p = .07$, $\eta^2 = .016$. Women spent significantly more time caregiving than men, $F(1,321) = 12.32$, $p = .001$, $\eta^2 = .037$, and suffered more strain, $F(1,328) = 15.72$, $p < .001$, $\eta^2 = .046$. There were no significant differences between the household occupational groups for the caregiving variables, although there was a trend for those from manual occupational households to report more hours spent caregiving per week, $F(1,314) = 2.97$, $p = .09$, $\eta^2 = .009$. The summary statistics for all the caregiving variables are presented in Table 1.

As might be expected, caregiving strain and burden were positively associated, $r(324) = .43$, $p < .001$. For the youngest, middle, and older cohorts, the correlations were $r(52) = .57$, $p < .001$, $r(154) = .47$, $p < .001$, and $r(114) = .33$, $p < .001$, respectively. It would appear that the association between strain and burden declines with age.

[Insert Table 1 about here]

3.2. S-IgA

For the sample as a whole, $\log_{10}$ S-IgA secretion rate differed between the age cohorts with older participants showing the lowest S-IgA, $F(1,1840) = 11.82$, $p < .001$, $\eta^2 = .013$. In addition, women had lower $\log_{10}$ S-IgA secretion rates than men, $F(1,1841) = 30.39$, $p < .001$, $\eta^2 = .016$; as did those from the manual occupational group, $F(1,1810) = 5.90$, $p = .015$, $\eta^2 = .003$. Secretion rates were lower in current smokers than ex smokers and never smokers, $F(2,1840) = 7.50$, $p = .001$, $\eta^2 = .008$. Assay batch also affected $\log_{10}$ S-IgA secretion rate, $F(3,1839) = 22.52$, $p < .001$, $\eta^2 = .035$, with higher values being recorded for the earlier batches. These effects parallel those reported previously in analyses of demographic variables, life events, and S-IgA in these data (Evans et al., 2000; Phillips et al., 2006).

3.3. Caregiving and S-IgA

In the initial ANCOVA, there were no significant main effects of caregiving status or age cohort on S-IgA secretion rate. However, there was a significant caregiving status by age cohort interaction effect, $F(2,1834) = 3.40$, $p = .03$, $\eta^2 = .004$. To explore this interaction further, separate ANCOVAs were conducted within the age cohorts. Caregivers showed significantly lower $\log_{10}$ S-IgA secretion rates than non caregivers, only in the eldest cohort, $F(1,577) = 6.55$, $p = .01$, $\eta^2 = .011$, (see Figure 1). In further analyses, the significant interaction between caregiving status and cohort survived adjustment for sex and household occupational group, $F(1,1801) = 3.22$, $p = .04$, $\eta^2 = .004$. It is possible that the interaction between caregiving status and cohort reflected variations in medication use. Seven, four, and five percent of the whole

sample reported that they were taking antihypertensive, anxiolytic, and antidepressant medications, respectively. The interaction effect was still significant following adjustment for whether or not participants were taking medication, $F(1,1829) = 3.18$, $p = .04$, $\eta^2 = .003$. In addition, the main effect for caregiving status in the eldest cohort remained significant following control for all three medications $F(1,572) = 5.82$, $p = .02$, $\eta^2 = .010$.

[Insert Figure 1 about here]

In hierarchical linear regression analysis within the caregivers, in which assay batch, saliva volume, and smoking type were entered at step 1, and the caregiving variables individually at step 2, a negative association between caregiving variables and $\log_{10}$ S-IgA secretion rate emerged. For caregiving strain, $\beta = -.09$, $t = 2.40$, $p = .02$, $\Delta R^2 = .009$, and total caregiving burden, $\beta = -.09$, $t = 2.24$, $p = .03$, $\Delta R^2 = .008$, such that higher caregiving scores were associated with lower S-IgA secretion rates. The outcomes of each step of the regression analyses are shown in Table 3. Time spent caregiving was not significantly associated with S-IgA secretion rate.

[Insert Table 2 about here]

We then conducted analyses entering batch, volume, and smoking status at step 1, the caregiving variables individually and age cohort at step 2, and the interaction between the caregiving variables and age cohort at step 3. Caregiving strain and burden remained negatively associated with S-IgA secretion rate in these models. There were significant interactions between age cohort and the caregiving variables. The regression statistics are presented in Table 3.

[Insert Table 3 about here]

These significant interactions were explored further by conducting regression analyses within each cohort. Higher caregiving strain and burden were each associated with lower $\log_{10}$ S-IgA secretion rates in the youngest, $\beta = -.25$, $t = 2.57$, $p = .01$, $\Delta R^2 = .055$ and $\beta = -.22$, $t =$

2.17, $p = .04$, $\Delta R^2 = .041$, respectively, and middle, $\beta = -.12$, $t = 2.05$, $p = .04$, $\Delta R^2 = .013$ and $\beta = -.18$, $t = 3.03$, $p = .003$, $\Delta R^2 = .027$, cohorts, but not in the eldest cohort, $\beta = .02$, $t = 0.28$, $p = .78$, $\Delta R^2 = .000$ and $\beta = .03$, $t = 0.41$, $p = .68$, $\Delta R^2 = .001$.

Subsequent regression analyses controlled for sex and household occupational group at step 2. The relationship between caregiving strain and S-IgA secretion rate was attenuated, although the interaction remained significant. The association with total caregiving burden and its interaction with cohort were both significant. The outcomes of each step of the regression analyses are shown in Table 4.

[Insert Table 4 about here]

3.4. Additional analyses

In a previous analysis of these data, we reported that life events stress was negatively associated with S-IgA secretion rates; the greater the stress, the lower the S-IgA (Phillips et al., 2006). These earlier analyses were limited to the middle and eldest cohorts, as they were the only ones to complete the life events measure. This was an inventory exploring participants' exposure to major life events in eight domains (health, marriage, relationships, deaths, work, housing, finance, and general) over the previous two years. Participants were confronted with 59 possible stressful events and asked to identify those that happened to them and, for each event, to specify on 5-point scales, how disruptive and how stressful the event was at the time of occurrence and now. Four measures of stress load were derived from the sums of the product of each life event experienced and its rated disruptiveness and stressfulness then and now. All four measures were negatively associated with S-IgA secretion rate. Accordingly, it might be argued that the present analyses are not yielding unique results. However, the life events inventory, available from the authors on request, contains no items explicitly relating to caregiving or its burden; the closest is probably the very general item 'other problems with relationships'. Nevertheless, for reassurance, we repeated the main analyses adjusting in turn for the four life events measures, as well as adjusting for assay batch, saliva volume, and smoking status. These analyses are necessarily restricted to the two older cohorts. Our previously reported results for caregiving

obtained. For the eldest cohort, caregivers continued to have significantly lower S-IgA secretion rates than non-caregivers, $F(1,576) = 6.61$ to 6.92, $p = .01$ to $.009$, $\eta^2 = .011$ to $.012$. Similarly, in the hierarchical regression analyses, caregiving strain for caregivers in the middle cohort continued to be negatively associated with S-IgA, following adjustment for the stress load scores, $\beta = -.14$, $t = 2.53$ to 2.58, $p = .01$, $\Delta R^2 = .019$ to $.020$. A similar picture emerged from the analyses of caregiving burden, $\beta = -.19$, $t = 3.23$ to 3.24, $p = .001$, $\Delta R^2 = .030$ to $.031$. Consequently, the association between caregiving stress and S-IgA would appear to be independent of any stress load imposed by general life events exposure and the current analyses yielded unique findings.

4. Discussion

A primary aim of the present analyses was to determine whether caregivers would have a lower S-IgA secretion rate than non-caregivers. Although the overall mean difference in secretion rate between caregivers and non-caregivers was not statistically significant, there was a significant interaction between caregiving status and age cohort. Caregivers in the eldest cohort had significantly lower S-IgA secretion rates than their age-matched non-caregiving counterparts. Thus, in line with our second hypothesis, it would appear that being a caregiver may pose a particular problem for immunity in older adults. In a previous study examining specific serum immunoglobulins (e.g. IgM and IgA) to latent viruses, no differences emerged between younger caregivers and age-matched controls (Pariante et al., 1997). There are a number of possible explanations for why older caregivers may be particularly susceptible. First, there is the issue of immunosenescence; the ageing immune system may simply be less able to deal with chronic stress. Others have recently postulated that psychological stress can exacerbate the effects of ageing on immunity and that older adults frequently suffer greater immunological consequences as a result of stress exposure (Graham et al., 2006). Consistent with this, a meta-analysis found that decreases in natural killer cell and lymphocyte activity are more often apparent in studies of older individuals (Segerstrom and Miller, 2004). Second, age variations in psychosocial context may also be important. For example, it has been argued that older caregivers may be less able to

withstand the rigors of caregiving due to their poorer psychosocial context, particularly reduced access to supportive social contacts (Vedhara et al., 2002).

To our knowledge, this is the first time that the chronic stress of being a caregiver has been shown to be associated with S-IgA. Accordingly, the general contention that the chronic stress of caregiving is associated with poor immune function (Cohen and Pollack, 2005; Kiecolt-Glaser et al., 1991; Kiecolt-Glaser et al., 1996; Kiecolt-Glaser et al., 1987; Mills et al., 2004) (Pariante et al., 1997) is given further weight by the present data. In addition, the present results suggest at least one possible explanation for the higher incidence and duration of infectious episodes in caregivers (Cohen and Pollack, 2005; Kiecolt-Glaser et al., 1991; Pariante et al., 1997). In a previous analysis of the impact of life events on S-IgA in the middle and eldest cohorts we reported a negative association between stress load and S-IgA secretion rate (Phillips et al., 2006). The present association between S-IgA and caregiving status would appear to be independent of any stressful life events effects. Stressful life events and caregiving may be phenomenally different. It has been argued that life events may be secondary in terms of health impact compared to the chronic stress afforded by caregiving (Pearlin, 1989).

The outcomes of the analyses within caregivers supported our third hypothesis; there was an association between caregiving load and S-IgA secretion rates, such that caregivers who experienced greater caregiver strain and burden had lower S-IgA secretion rates. This result is consistent with the outcomes of previous studies that have examined caregiver burden in this context (Cohen and Pollack, 2005; Gennaro et al., 1997; Pariante et al., 1997). Within-cohort analyses indicated that the association between strain, burden, and S-IgA was statistically significant for the younger and middle cohorts but not for the eldest cohort. One reason for this may be reduced statistical power, which drops substantially from the overall to the within-cohort analyses. However, it should be noted that it was the youngest not the eldest cohort that had the fewest caregivers. A second possibility is that the impact of caring in older participants may simply be more homogeneous; i.e., it is whether or not they are caregiving that is critical for immunity not the precise extent of the strain and burden it imposes. It is worth noting in this context that the correlation between caregiving burden and strain was smallest in the eldest cohort. In addition, the older caregivers showed the lowest variability in S-IgA secretion rate, making associations more difficult to find.

The present study has several limitations. First, only one measure of S-IgA was taken, a concern given circadian variation (Hucklebridge et al., 1998). However, more comprehensive monitoring was not practical in a large community study. Further, S-IgA appears to be stable from around 3 hours after awakening (Hucklebridge et al., 1998) and early morning testing was rare. S-IgA also shows good test-retest reliability over days and weeks (Ring et al., 2002; Willemsen et al., 1998). Second, we are unable to shed much light on the mechanisms underlying the observed associations between caregiving stress and S-IgA. However, by statistical adjustment, we were able to discount the possibility that they were driven by variations in saliva volume. This is important because it allows us to eliminate a parsimonious but relatively less interesting explanation, i.e. that for older caregivers and those with a high caregiving burden, the glands simply release less saliva into the mouth. Rather, the present analyses suggest that caregiving stress either decreases IgA production by the local plasma cells or reduces the efficiency with which S-IgA is transported from the glandular interstitium into saliva. However, it is difficult to identify pathways beyond this level in large scale community studies of this sort. Nevertheless, clues to the sensitivity of S-IgA to cytokines and hormones can be found in previous small scale laboratory studies. For example, secretion of the cortisol has been reported to correlate with secretion of S-IgA (Hucklebridge et al., 1998). Further, the poly-Ig-receptor, which is expressed in epithelial cells and influences the transportation and secretion of S-IgA, is regulated by both hormones (e.g. glucocorticoids and adrenergic neurotransmitters) (Sabbadini and Berczi, 1995) and cytokines (e.g. IFN-γ and TNF), (Brandtzaeg et al., 1992). In addition, hormone and cytokine profiles have been found to differ between caregivers and non-caregivers (Kiecolt-Glaser et al., 1996; Miller et al., 2002; Vedhara et al., 1999). Thus, taken together, these findings suggest two possible pathways through which caregiving may alter S-IgA secretion. Third, we should concede that the effect sizes reported here are small. Nevertheless, they are larger than those we reported previously for life events and S-IgA (Phillips et al., 2006). In addition, the present associations are robust and survive correction for a range of possible confounders rarely, if ever, considered in S-IgA research. Finally, these analyses relied on a somewhat crude composite index of caregiving burden, constituted from fairly simple metrics. However, despite this, associations still emerged between the derived variables and S-IgA in the hypothesised direction.

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In summary, older caregivers showed lower S-IgA secretion rates than age-matched controls, which could not be accounted by variations in saliva volume. Further, S-IgA secretion rate decreased as caregiver burden increased. We believe this is the first study to implicate caregiving stress in mucosal immunity. As such, our findings reinforce the view that this particular chronic stress exposure has varied and profound effects on immune function.

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Table 1: Caregiving variable means (SDs) by age cohort, sex, and household occupational status.

	Youngest	Middle	Eldest	Men	Women	Manual	Non-manual
Time spent caregiving*	7.4 (9.68)	8.1 (10.85)	13.8 (19.93)	7.7 (11.72)	11.7 (16.34)	10.8 (14.55)	9.1 (15.35)
Caregiving strain rating	2.0 (1.21)	2.5 (1.74)	2.4 (1.25)	2.0 (1.17)	2.7 (1.64)	2.4 (1.47)	2.5 (1.57)
Types of help given	2.3 (1.53)	2.7 (1.86)	2.7 (2.40)	2.5 (1.88)	2.7 (2.11)	2.7 (1.90)	2.6 (2.19)
Composite residence score	2.4 (0.63)	2.4 (0.88)	2.3 (0.80)	2.4 (0.82)	2.3 (0.81)	2.3 (0.83)	2.4 (0.81)
Total caregiving burden	5.9 (4.50)	7.1 (8.26)	6.8 (8.02)	6.4 (6.33)	7.0 (8.40)	6.8 (6.09)	6.9 (9.32)

* hours per week

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Table 2: Regression analyses predicting $\log_{10}$ S-IgA secretion rate by caregiving strain and burden among caregivers.

		β	t	*p*	ΔR^2
Step1:	Assay batch	-.13	3.34	.001	
	Saliva volume	.69	17.87	<.001	
	Smoking status	-.06	1.47	.14	.509
Step 2:	Caregiving strain	-.09	2.40	.02	.009
Step1:	Assay batch	-.13	3.39	.001	
	Saliva volume	.69	17.66	<.001	
	Smoking status	-.06	1.54	.12	.510
Step 2:	Total caregiving burden	-.09	2.24	.03	.008

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Table 3: Regression analyses predicting $\log_{10}$ S-IgA secretion rate by age cohort, caregiving strain and burden, and the interaction between the caregiving variables and age cohort, in caregivers.

		β	t	*p*	ΔR^2
Step1:	Assay batch	-.13	3.25	.001	
	Saliva volume	.69	17.74	<.001	
	Smoking status	-.06	1.58	.12	.509
Step 2:	Age cohort	-.08	2.00	.05	
	Caregiving strain	-.09	2.20	.03	.014
Step 3:	Strain x Cohort	.24	2.29	.003	.008
Step1:	Assay batch	-.13	3.22	.001	
	Saliva volume	.69	17.63	<.001	
	Smoking status	-.07	1.69	.09	.510
Step 2:	Age cohort	-.09	2.13	.03	
	Total caregiving burden	-.09	2.29	.02	.014
Step 3:	Burden x Cohort	.28	2.67	.008	.010

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Table 4: Regression analyses predicting $\log_{10}$ S-IgA secretion rate by caregiving strain and burden among caregivers, adjusting for potential confounding variables.

		β	t	*p*	ΔR^2
Step1:	Assay batch	-.13	3.19	.002	
	Saliva volume	.69	17.33	<.001	
	Smoking status	-.06	1.56	.12	.503
Step 2:	Sex	-.10	2.50	.01	
	Occupational group	.00	0.03	.98	.010
Step 3:	Age cohort	-.08	2.03	.04	
	Caregiving strain	-.07	1.64	.10	.011
Step 4:	Strain x Cohort	.24	2.26	.02	.008
Step1:	Assay batch	-.13	3.16	.002	
	Saliva volume	.69	17.21	<.001	
	Smoking status	-.07	1.67	.10	.504
Step 2:	Sex	-.10	2.53	.01	
	Occupational group	.00	0.04	.97	.010
Step 3:	Age cohort	-.09	2.13	.03	
	Total caregiving burden	-.08	2.10	.04	.013
Step 4:	Burden x Cohort	.29	2.65	.008	.010

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Figure 1. Mean (SE) $\log_{10}$ S-IgA secretion rate for caregivers and non-caregivers by age cohort, controlling for saliva volume, assay batch and smoking status.

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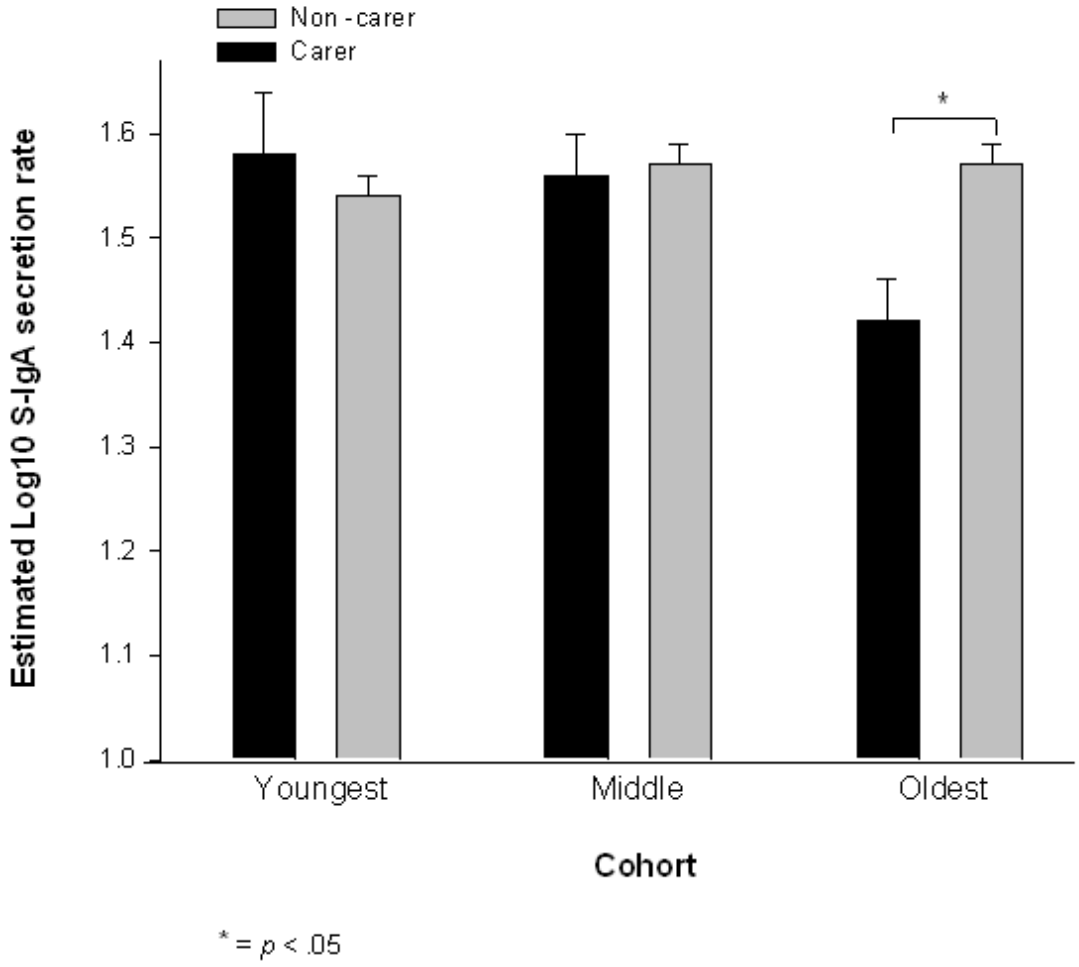

* = $p < .05$