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Depression in caregivers of demented patients is associated with altered immunity: impaired proliferative capacity, increased $CD8^+$, and a decline in lymphocytes with surface signal transduction molecules ($CD38^+$) and a cytotoxicity marker ($CD56^+$ $CD8^+$)

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SUMMARY

Changes in relevant immune parameters, including function, were found to be associated with depression in elderly caregiver wives of demented patients. We studied the relationship between immune cell phenotype and T cell proliferative capacity of such caregivers to levels of stress and depression over the course of a support group intervention. The data indicate the strongest association between depression (of all stress parameters) and impaired T cell proliferative capacity. Depression was also most strongly (of stress parameters) associated with a shift in T cell populations with an increase in $CD8^+$ T cells, and a reduced percentage of $CD38^+$ cells in both $CD8^+$ and $CD4^+$ T cell populations. Since CD38 is a signal transduction factor, it was interesting that a decreased percentage of $CD38^+$ cells correlated with impaired T cell function (proliferation). Another significant difference was the reduction in natural killer (NK) cells as well as the percentage of the $CD56^+$ component of the $CD8^+$ population. This latter subset is important in MHC-unrestricted cytotoxicity, and has been found expanded in healthy centenarians. This study shows that both chronic stress, and depression in particular, and age have deleterious effects on T cells, and together could significantly contribute to the higher risk of disease and mortality associated with being a caregiver of a demented individual.

Keywords depression immune dysfunction CD38 CD56 ageing

INTRODUCTION

Common belief in lay publications is that people get sick when they are stressed [1]. Anecdotal experience suggests that premature illness or death among the healthier spouse in situations of long-term caregiving for a chronically ill spouse is not uncommon. There is significant evidence that acute and chronic stress results in change in immune function, which may be mediated by brain corticotrophin-releasing hormone (CRH) and the autonomic nervous system [1]. Stress models in mice have suggested an increase in CRH and impaired natural killer (NK) activity, T cell mitogenic response and impaired T cell-dependent antibody response which was blocked by CRH antagonist [2,3]. Most studies on chronic stress have shown consistent impairment of NK cell activity, including a rat study using overcrowding, which reported an increase in tumour growth [4]. However, another murine model of chronic stress using noise exposure suggested adaptation and even enhancement of immune function in chronic stress (like hormesis: the stimulating effect of subinhibitory concentrations of any toxicity on an organism), in comparison with acute stress, with regression of tumours [5]. Hence, the impact of stress on health may be related to specific components of the stress, including a subject's ability to cope. Marital disruption has been identified as one of the most significant life stressors, and is associated with increased mortality, particularly from pneumonia, tuberculosis, heart disease and some cancers [6,7]. In a study investigating the impact of bereavement on immunity, no statistical difference in immune parameters (total lymphocyte count, NK activity, T cell proliferative capacity, T cell subsets) was found between widows and age-matched controls. However, the subset of widows with a diagnosis of major depression demonstrated impaired NK activity and mitogenesis [8]. Depression, including the aged as a cohort, has been associated with reduced proliferation to mitogen, with low cytokine

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production (IL-2 and IL-4, and interferon-gamma (IFN-γ)), as well as impaired NK activity which was not reversed by the exogenous addition of IL-2 or IFN-γ [9–16]. Caregivers of demented patients, in particular, have been reported to have higher mortality, depression, anxiety and social isolation than age-related peers, which is expected given the combination of chronic stress, disruption of marital relationship, and perhaps some capacity of bereavement [17]. A study specifically in caregivers of Alzheimer patients suggested impairment of immunity to control latent viral infection, with increases in antibody titres to Epstein–Barr virus (EBV) viral capsid antigen, and a reduction in $CD4^+$ T cells, but no change in $CD8^+$ cells. This study, however, included children caregivers and did not differentiate the specific impact of depression on immune function [18]. Another study reported that impaired NK activity in caregivers as well as in depression was associated with an increase in neuropeptide Y (NPY), suggesting a relationship between stress-induced increased catecholamines and central nervous system down-regulation of immune response [19]. The relationship between stress and advanced age could be synergistic and may result in a drop below threshold immune competence levels, contributing to the increased morbidity and mortality in this population [19].

We had the opportunity to study the relationship between a number of psychological stress parameters as well as to conduct an in depth phenotypic analysis of immune cell subsets and T cell proliferative function in a small group of caregiver wives of demented patients. The goal of this pilot study was to identify trends between specific stress parameters with specific immune parameters over the course of a support group intervention, that would focus analysis in the identification of the mechanisms of immune dysfunction in this high risk group.

SUBJECTS AND METHODS

Subjects were recruited by contacting potential subjects in the Geriatric Outpatient Clinic, the Neurobehaviour and Dementia Clinics at the VAMC West Los Angeles, as well as the UCLA Alzheimer's Disease Centre. Eleven wives who lived with demented spouses were recruited, with a mean age $70{\cdot}0 \pm 5{\cdot}7$ years. All were primary caregivers and none had a significant history of alcohol use, a prior history of a psychiatric disturbance, or a history of medications or illness known to be associated with significant immune depression. All study subjects had stable medical conditions with no change in overall health status or change in medications through the course of study. Seven subjects used a variety of cardiovascular agents, but none used beta adrenergic antagonists. Four subjects were receiving oestrogen replacement, three thyroxine, two were on antidepressants, and three used intermittent benzodiazipines for anxiety and/or sleep. After giving consent, subjects received an initial battery of psychologic tests: the Beck Depression and Anxiety Inventories, Hamilton Depression Scale, Geriatric Depression Scale (GDS), Folstein Mini-mental Status Exam (MMSE), Zarit's Caregiver Burden Scale (ZAR), Lubben's Social Network Scale, UCLA Domain of Caregiver Appraisal, and the Symptom Checklist-90-Revised (SCL-90-R). Cut-offs for levels of depression or stress used have been tested and validated for all stress parameters. The demented spouses all received a MMSE, GDS, and the Hamilton Depression Inventory. This battery of tests was repeated at the end of the 8-week intervention and at 1 month post-intervention follow up. The intervention consisted of a minimum of 1·5 h of support group therapy weekly for 8 weeks focused on building coping skills through education, shared experience and a supportive environment to address each caregiver's current concerns.

Subjects also had 60 ml of blood drawn to measure immunological parameters at the onset of the intervention (week 0 and week 1), at mid intervention (week 5), at the conclusion of the intervention (week 8), and at 1 month follow up (week 12). Lymphocyte phenotype characterization was provided by the CIRID Laboratory at UCLA on whole blood samples in EDTA anticoagulant, and peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a Histopaque density gradient (Sigma, St Louis, MO). Samples were also utilized for functional T cell analysis of proliferative capacity (to both phytohaemagglutinin (PHA), and anti-CD3). Three to six young, healthy, female controls (mean age $34{\cdot}0 \pm 7{\cdot}4$ years) were utilized to help standardize assay results. All blood samples were obtained between 9 and 11 a.m.

For immunophenotyping, PBMC were distributed among 12×75 mm plastic tubes, with 5×10^5 cells/tube, containing the following MoAb combinations (Becton Dickinson Immunocytometry Systems, San Jose, CA): $CD3^+/CD56^+$ 16/CD8, HLA-DR/CD38/CD8, CD71/CD25/CD8, CD57/CD56/CD8, HLA-DR/CD38/CD4, CD71/CD25/CD4, and CD71/CD26/CD19. In each case the first MoAb (FL1) was labelled with FITC, the second (FL2) with PE, and the third (FL3) with peridinin chlorophyll protein (PerCP). Tubes were incubated for 15 min (room temperature), washed, and resuspended in 0·25 ml fixation buffer (1% paraformaldehyde in $1\times$ PBS). Cells were analysed using the FACScan flow cytometer (Becton Dickinson). FL1 (FITC) was measured using a 530/30 nm band-pass filter, FL2 (PE) with a 585/42 nm band-pass filter, and FL3 (PerCP) with a 650 nm long-pass filter. Anti-CD45 FITC and anti-CD14 PE served as markers for validating lymphocyte scatter gating. The fluorescence negatives were then defined by using isotype control antibodies and compensations adjusted by $CD3/56^+$ 16/8 tube. List mode data were collected for 10 000 events in the total gate. Lymphocytes were identified by gating on forward (low angle) and 90° (wide angle) light scatter parameters, and verified by use of LeucoGATE Simultest (Becton Dickinson). To determine the percentage of $CD4^+$, $CD8^+$, or $CD19^+$ lymphocytes co-expressing one or both of the additional markers under analysis, a gate was set that included only FL2-positive cells. The absolute number of cells within a subset was determined by multiplying the absolute lymphocyte count by the percentage cells positive for that marker(s). The absolute lymphocyte count was obtained from the differential counts performed by Coulter counter analyses.

For T cell functional analysis, PBMC isolated by density centrifugation as described above were then placed (1×10^6 cells/ml) in 96-well plates (Becton Dickinson) and stimulated with anti-CD3 (0·25 μg/ml; Dakopatts, Carpinteria, CA) and PHA (5 μg/ml; Sigma) and cultured for 72 h. Both mitogens were at doses that have produced maximal proliferative response in both younger adult and elderly subjects in this laboratory. Proliferative capacity was determined by pulsing the cell cultures with 0·4 μCi tritiated thymidine (New England Nuclear, Boston, MA) 8 h before harvesting cells. The incorporation of radiolabel was then determined by placing the cells harvested on filter paper in a beta counter (Beckman, Fullerton,

CA). Counts for the study subjects were expressed as a percentage of the mean proliferative capacity for the young female subjects. These functional and phenotypic immune parameters were measured five times over a 12-week period. The data, correlations and comparisons represent both at the time of entry into the study and the mean of the five measures of each parameter per person, as explained in more detail in Results. Statistical analysis included linear regression, Spearman's rank correlation and Student's *t*-test, as appropriate.

RESULTS

T cell function versus *phenotype*

At the start of the study, the mean percentage proliferation (standardized to the young controls) to anti-CD3 of the caregiver support group correlated inversely with the mean percentage of CD8$^+$ cells ($r = -0.853, P < 0.0009$), but not with the mean percentage of CD4$^+$ cells ($r = 0{\cdot}558$, $P < 0{\cdot}07$; see Fig. 1a). Of interest, there was no correlation found between age of the caregivers and proliferation (age *versus* PHA $r = 0{\cdot}041$, $P > 0{\cdot}9$; and *versus* anti-CD3 $r = -0{\cdot}439$, $P > 0{\cdot}17$); hence, certain phenotypic (and stress) characteristics had an association with T cell function, while age did not. There was also significant correlation of proliferation to anti-CD3 with the mean percentage of CD38$^+$ cells, including both CD38,8$^+$ ($r = 0{\cdot}705$, $P < 0{\cdot}02$) and CD38,4$^+$ cells ($r = 0{\cdot}783$, $P < 0{\cdot}005$; see Fig. 1b). Proliferation to PHA did correlate with the mean per cent CD4$^+$ cells ($r = 0{\cdot}797$, $P < 0{\cdot}004$), but not with the per cent CD8$^+$ cells (data not shown).

Stress parameters versus *immune parameters*

The GDS correlated the best of all stress parameters with immune parameters. While limited sample size does not exclude significant associations, there were no other significant correlations by logistic regression or Spearman's rank correlation between other stress parameters and immune parameters. At the start of the intervention (week 1), the level of depression (GDS) correlated inversely with proliferation to anti-CD3 ($r = -0{\cdot}614$, $P < 0{\cdot}05$) and inversely to the number of activated CD8,38$^+$ cells ($r = -0{\cdot}636$, $P < 0{\cdot}02$), but depression correlated directly with the per cent CD8$^+$ cells ($r = 0{\cdot}633$, $P < 0{\cdot}04$). The GDS score had no correlation with proliferation to PHA ($P > 0{\cdot}3$) or the percentage CD4$^+$ cells ($P > 0{\cdot}6$). In other words, the more depressed, the less proliferation to anti-CD3, the higher the percentage CD8$^+$ cells, but the lower percentage activated CD8,38$^+$ cells (data not shown).

In addition, by comparing the means of immune parameters at times when caregivers had evidence of depression, defined as a GDS $\geqslant 11$ [21] at any time at weeks 1, 8, and 12 ($n = 11$), *versus* those time points without evidence of depression (GDS < 11, $n = 18$), clear associations of immune parameters with depression were identified. While there was fluctuation in stress parameters over the course of the study, with a trend toward increasing stress at 8 weeks and a decline thereafter,

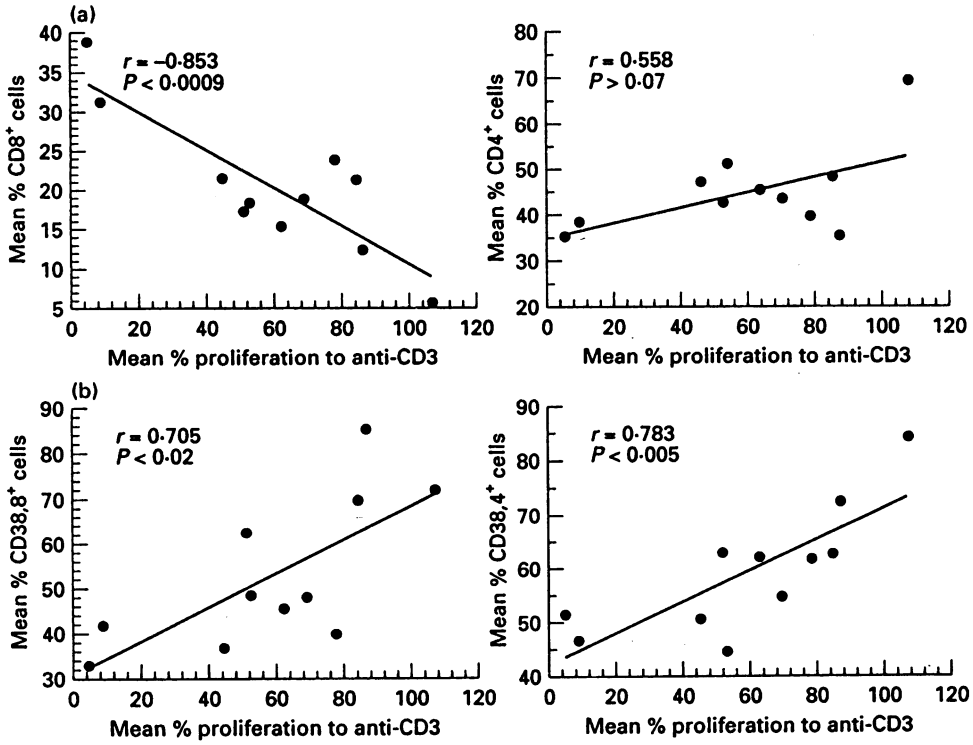

Fig. 1. (a,b) Correlation of proliferation with immune cell phenotype (a, *versus* per cent CD4$^+$ and per cent CD8$^+$; b, *versus* per cent CD38,8$^+$ and per cent CD38,4$^+$). This is a correlation of the mean of five measures over a 12-week period for each of 11 caregivers. The proliferation to anti-CD3 (0·25 μg/ml) in a 72-h tritiated thymidine uptake assay is expressed as a percentage of young controls (mean ct/min 103 315 $\pm$ 4599) assayed at the same time. Immune cell phenotype is expressed as a percentage of cells (see Subjects and Methods).

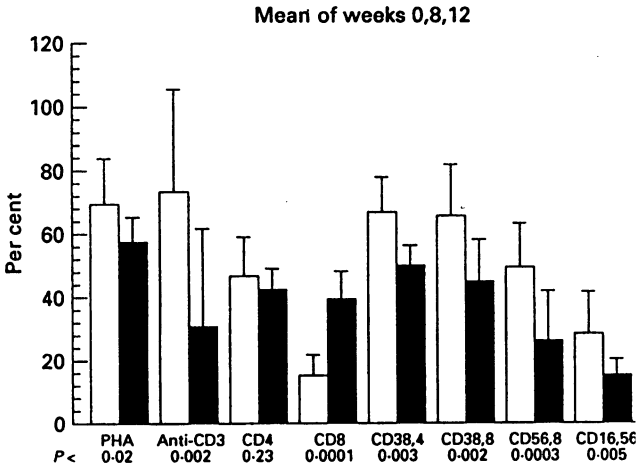

Fig. 2. Difference in immune parameters of non-depressed (□) *versus* depressed (■) caregivers. This is a comparison of the mean ± s.d. of three measures of immune parameters of non-depressed caregivers (Geriatric Depression Scale (GDS) < 11, $n = 18$) *versus* the mean ± s.d. of immune parameters of depressed caregivers (GDS ⩾ 11, $n = 11$). Statistical significance of differences between groups as per Student's *t*-test. Again, proliferation is expressed as per cent proliferation of the young controls, and phenotype is expressed as mean per cent of cell subtypes.

shift in depression scores did not cross thresholds of severity except in one subject, with a shift in GDS score large enough to move from the mild depression level to no depression; this subject was left out of the analysis for association of depression with mean measures of phenotype. Caregivers with evidence of depression showed less T cell proliferation to both PHA ($P < 0{\cdot}02$) and anti-CD3 ($P < 0{\cdot}002$). There was no significant change in per cent $CD4^+$ cells, but an increase in per cent $CD8^+$ cells ($P = 0{\cdot}0001$), as shown in Fig. 2. Depressed caregivers also had significantly lower percentage 'activated' T cells that express the $CD38^+$ marker, both for the $CD8^+$ ($P < 0{\cdot}002$) and $CD4^+$ subset ($P < 0{\cdot}003$). The lower percentage $CD38^+$ cells occurred primarily in the HLA-DR^- subset (see Fig. 3).

The depressed group also showed a significant decrease in $CD56,8^+$ cells ($P < 0{\cdot}003$) and $CD56,16^+$, $CD3^-$ NK cells ($P < 0{\cdot}005$). The decline of $CD56,8^+$ cells in the depressed group was most pronounced in the $CD56^+$ 57^- subset (see Fig. 4). Although there was no significant shift in the total $CD57,8^+$ subset, the depressed group did show a significant expansion of the $CD57^+$ 56^- subset. In this limited sample size, no correlation was found with either T cell function or stress parameters to many of the immune cell phenotypes, including CD57, CD71 and activated CD4 cells ($CD25,4^+$), and no clear relationship between medication use and immune cell phenotype or function was identified.

Analysing the data using absolute cell numbers instead of cell percentage resulted in the loss of significance of some associations (CD38 and CD56,8 subsets) because of increased variability in absolute cell number, but an increased significance in $CD57,8^+$ subset association (with higher absolute number of $CD57^+$ cells associated with depression). Of $CD8^+$ cells, there was a decrease in the per cent $CD38^+$ in the depressed group, but an increase in the absolute number of $CD38,8^+$ cells.

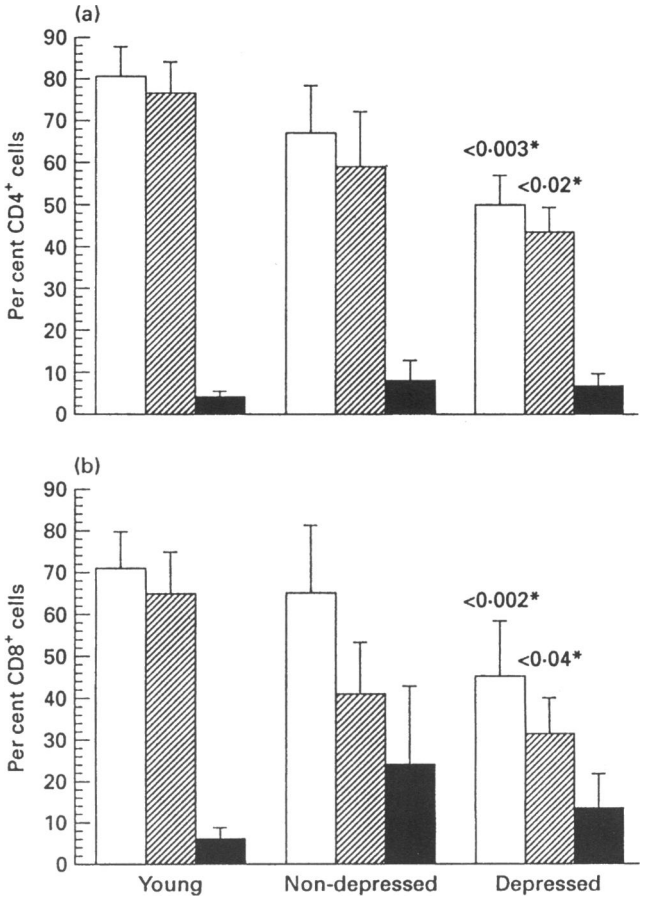

Fig. 3. Shift in CD38 T cell subsets for (a) $CD4^+$ T cells, and (b) $CD8^+$ T cells. This is a comparison for mean ± s.d. of cell phenotype of depressed *versus* non-depressed caregivers at the three different time points studied. DR refers to the percentage of $CD4^+$ or $CD8^+$ cells that were also HLA-DR^+. □, Total $CD38^+$; ▨, $CD38^+DR^-$; ■, $CD38^+DR^+$. **P versus* non-depressed.

Age-related changes in immune phenotype not clearly due to depression

Age-related differences in immune cell phenotype (not clearly associated with depression) were found, including a reduced percentage of $CD4^+$ cells but a higher percentage of NK ($CD16,56^+$,$CD3^-$) cells. There was an age-related increase in the percentage $CD57,8^+$ subset that was mostly in the $CD56^-$ subset (which is very different from the shift seen in depressed caregivers). In both the $CD8^+$ and $CD4^+$ cells, there was a decreased percentage of $CD38^+$ cells (predominately in DR^- subsets), while there was an age-associated increased percentage of DR^+ subsets in the caregivers (see Figs 3 and 4). There was also a significant age-related increase in the percentage of $CD4^+$ cells expressing CD25 (IL-2 receptor), with $53{\cdot}7 \pm 8{\cdot}9$ in caregivers *versus* $37{\cdot}3 \pm 5{\cdot}7$ in young ($P < 0{\cdot}0007$).

DISCUSSION

Several interesting observations from this pilot study suggest potential mechanisms of the immunosuppression that is associated with chronic stress, and specifically depression. A reduced T cell proliferative capacity to anti-CD3 and PHA

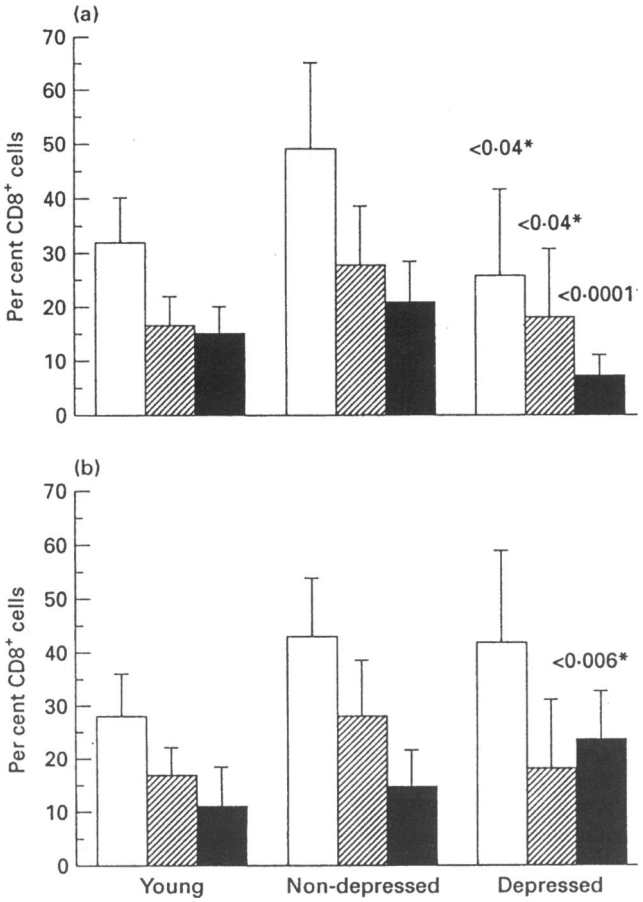

Fig. 4. Shift in CD56 (a) and CD57 subsets (b) of CD8$^+$ cells. This is a comparison of the shift in mean percentage ± s.d. of CD56 and CD57 expression in depressed *versus* non-depressed caregivers. (a) □, Total CD56$^+$; ▨, CD57$^+$56$^+$; ■, CD57$^-$56$^+$. (b) □, Total CD57$^+$; ▨, CD57$^+$56$^+$; ■, CD57$^+$56$^-$. **P versus* non-depressed.

was found in stressed and depressed caregivers. This functional deficit was associated with an increase in the percentage of CD8$^+$ T cells. In the present study, the percentage of CD4$^+$ cells did not correlate with proliferation. This may suggest that immune dysfunction associated with depression is due more to expansion of some CD8$^+$ subsets, rather than the deletion of CD4$^+$ subsets.

This study also suggests an important association of the CD38 glycoprotein surface marker with T cell function that may be altered by the psychological stress associated with depression. An association was identified between depression, reduced proliferative capacity and a reduced percentage of CD38$^+$ cells in both the CD4$^+$ and CD8$^+$ populations. Evidence indicates that CD38 function is involved in a unique pathway of signal transduction, cell adhesion, and cell cycle [22–24]. Studies have reported an over-expression of CD38 mRNA in proliferating T cells [25], and triggering the CD38 receptor with CD38 MoAb has been shown to induce IL-1, tumour necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6 [26]. It has also been suggested that CD38 may function as an ectoenzyme that becomes internalized and acts as a second messenger by increasing intracellular calcium, via the synthesis of cyclic ADP ribose (cADPR) [27]. An increase in CD38 expression has been found in some viral infections, with elevated CD38 expression associated with chronic fatigue syndrome, and as an early predictor of clinical deterioration in HIV infection that may predate a fall in CD4 counts [28–31]. This study further supports the importance of CD38 in proliferative capacity. The low CD38$^+$ cell percentage associated with depression concurs with a previous study involving chronic fatigue syndrome, where a control population of depressed individuals without evidence of chronic fatigue syndrome also had low percentage of CD38$^+$ T cells [28]. To define best the causal relationship between stress/depression, shift in immune cell phenotype and function, these parameters need to be measured over time in addition to following viral titres such as to EBV or measures for reactivated virus in serum, such as by quantitative polymerase chain reaction. Regardless of causality, CD38 may be a good marker to identify elderly subjects with an impaired ability to shift cells into cell cycle.

Other activation markers, primarily in CD8$^+$ subsets, have generally been found to increase with advanced age, including DR, CD56 and CD57 [32–34]. These subsets are expanded in Down's syndrome patients, especially those of advanced age, and may be associated with the more profound age-related immune dysfunction seen in that population [34]. It is unclear whether this is a consequence of exposure to viruses or other pathogens, is a component of immune dysregulation, or is an effort to compensate for thymic involution with an expansion of MHC unrestricted cytolytic (CD56,8$^+$) cells. Expansion of CD57,8$^+$ cells has also been reported in patients with rheumatoid arthritis, Crohn's disease, transplant patients, HIV and cytomegalovirus (CMV) infection [35–38]. Further subdivision of the CD57,8$^+$ subset in normal, healthy individuals has revealed important functional differences [38]. Subdivision by the relative brightness (high *versus* low) of CD8 expression has revealed that the CD57$^+$,8^{hi} subset is associated with prior CMV exposure and suppresses T cell proliferation to pokeweed mitogen [39]. Expansion of CD57,8$^+$ alveolar cells from bronchial alveolar lavage has been found in HIV infection, and the level of expansion correlates with disease progression [40]. Furthermore, the CD57,8$^+$ cells blocked cytotoxic T lymphocyte (CTL) activity, which did not require cell-to-cell contact and was not due to TNF-α, TNF-β, IFN-α, IFN-γ, transforming growth factor-beta (TGF-β), or prostaglandin. Therefore, the overall age-related expansion of CD57$^+$ cells may contribute to impaired CMI, including a poor response to immunization in the elderly population in general. Furthermore, the expansion of the CD57$^+$,56$^-$ subset observed in the depressed caregivers in this study, the apparent most suppressive CD57 subset [37,38], may further contribute to immune dysfunction associated with depression.

The finding that the CD56$^+$ subset in CD8$^+$ T cells is diminished in the depressed caregivers in comparison with the non-depressed caregivers may be important. This subset of T cells has been found to show strong tumour lytic activity [41,42], and may be very important cells in host defence and tumour surveillance in aged individuals [33,39]. The consequence of diminished expansion of this subset, and whether these changes could be reversed by treatment of stress and or depression, should be studied.

These data suggest the depression associated with the chronic stress of caregiving is associated with immunomodulation, which may be mediated centrally by increased secretion of

CRH, with subsequent increase in NPY and alteration in immunity. Alternatively, a symptom common to depression, such as disrupted sleep (psychologically related to stress, or physically related to altered sleep of the demented spouses), could be specifically involved in the process, with an association only to depression. Other potentially confounding variables need to be studied in larger sample sizes, and over time, particularly relating to the use of medications including oestrogen and thyroid replacement, beta adrenergic antagonists, sedatives and antidepressants. These data suggest that the disease risk associated with the stress and depression of being a caregiver is associated with an increase in total $CD8^+$ T cells or a drop in the percentage of $CD38^+$ (both $CD4^+$ and $CD8^+$) T cells. The associated decline in T cell function, as measured by proliferative capacity, may be due to a shift in cytokines resulting from diminished expression of the CD38 surface receptor. The proinflammatory cytokines IL-1, TNF-α, GM-CSF or the pleotropic cytokine IL-6, all of which have been shown to be induced by stimulation of the CD38 receptor [22], could be involved in the mechanism of the associated reduced proliferation. The other significant finding was the associated decline of the $CD56,8^+$ subset and expansion of the specific $CD57^+$ $CD56^-$ subset in depressed individuals. This may impair tumour surveillance and/or defence against viral infections, given the apparent increased importance of this MHC-unrestricted subset (the $CD56,8^+$ subset) in the face of thymic involution.

While this study was limited by sample size, it suggests that depression, as measured by the GDS, as a component of the chronic stress of wives providing primary care to demented spouses, has the strongest association with altered immunity in comparison with measures of stress such as anxiety, perceived burden, level of social network or symptom checklists. A larger sample size and longitudinal data with treatment of stress in caregivers, particularly treatment of depression, and an adequate elderly 'non-caregiver' control population will be necessary to limit the confounding effects of the high prevalence of disease and medication use in this group of elderly caregivers. Stress parameters should also include specific measures of disruption of sleep, of both the caregivers and the demented spouse. Ultimate identification of causal relationships between specific components of stress and altered immunity will allow targeted therapy that should reduce the risk of infection.

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