

Slowing of wound healing by psychological stress

Janice K Kiecolt-Glaser, Phillip T Marucha, William B Malarkey, Ana M Mercado, Ronald Glaser

Summary

There is evidence that psychological stress adversely affects the immune system. We have investigated the effects of such stress, caused by caring for a relative with Alzheimer's disease, on wound healing.

We studied 13 women caring for demented relatives (mean age 62·3 [SE 2·3] years) and 13 controls matched for age (60·4 [2·8] years) and family income. All subjects underwent a 3·5 mm punch biopsy wound. Healing was assessed by photography of the wound and the response to hydrogen peroxide (healing was defined as no foaming). Wound healing took significantly longer in caregivers than in controls (48·7 [2·9] vs 39·3 [3·0] days, $p < 0·05$). Peripheral-blood leucocytes from caregivers produced significantly less interleukin-1 β mRNA in response to lipopolysaccharide stimulation than did controls' cells.

Stress-related defects in wound repair could have important clinical implications, for instance for recovery from surgery.

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Introduction

Cellular immunity has an important role in the regulation of wound repair. Proinflammatory cytokines such as interleukin-1, interleukin-8, and tumour necrosis factor (TNF) help to protect against infection, prepare injured tissue for repair, and enhance phagocyte recruitment and activation.¹ Furthermore, cytokines released by the recruited cells regulate the ability of fibroblasts and epithelial cells to remodel damaged tissue.^{1,2} Dysregulation of cytokines can impair wound healing.²

Animal and human studies have shown that stress can affect many features of cellular immune function, including cytokine production.³ For example, relatives who provide long-term care for a patient with Alzheimer's disease report high levels of stress as they attempt to cope with patients' difficult behaviour,⁴ and this chronic stress has immunological consequences. McCann⁵ found much poorer responses to delayed hypersensitivity skin testing in 34 spouses caring for such patients than in 33 matched non-caregivers; in fact, in comparison with normal age and sex standards, 50% of the caregivers were totally or relatively anergic, versus only 12% of non-caregivers. In other studies caregivers showed less enhancement than controls of natural-killer-cell activity in response to two cytokines that stimulate natural-killer-cell lysis, γ -,interferon and interleukin-2.^{6,7} Caregivers showed poorer proliferative responses to two mitogens than did controls.⁸

In our study of antibody and virus-specific T-cell responses to an influenza virus vaccine (to be published elsewhere) caregivers showed significant deficits relative to controls. Caregivers were less likely to show a satisfactory increase in antibody titre 4 weeks after vaccination, they had lower in-vitro interleukin-1 β responses to stimuli, and their peripheral-blood leucocytes produced less interleukin-2 in response to vaccine (antigen) stimulation. These findings suggest that caregivers are more vulnerable than age-matched non-caregivers to influenza and, potentially, to other infectious agents.⁹ Because the immune system has an important role in wound healing, we sought to find out whether there is any difference between caregivers and controls in the rate of healing of a standard wound or in proinflammatory cytokine response.

Subjects and methods

Caregivers (13) and control subjects (13) were recruited by means of announcements in hospital and university newspapers, notices in senior citizens' centres, and referrals from other participants. Caregivers and non-caregivers (all healthy women) were studied simultaneously, with groups matched for age and income. Reasons for exclusion were diabetes, peripheral vascular disease, previous difficulties with wound healing, use of antiinflammatory treatment or other drugs with obvious immunological consequences, or immunologically related health problems (eg, cancer, autoimmune disease, or recent surgery). The Ohio State University Biomedical Research Review Committee approved the project; before participation all subjects gave written informed consent.

The subjects were aged 47-81 years; the mean age was 62·3 (SE 2·3) years for caregivers and 60·4 (2·8) years for controls. The average family income for both groups was \$20 000 to \$30 000. The groups were not matched for marital status. Ten caregivers were married and three divorced or widowed; four control subjects were married, four divorced, two widowed, and three single. The inclusion of a greater proportion of unmarried control subjects worked against confirmation of the experimental hypotheses, because married people have lower rates of morbidity and mortality, as well as better immune function.^{3,10}

Caregivers had been providing care for a mean of 7·8 (0·6) years. Nine were caring for their husbands and four for their mothers. They spent an average of 6·7 (1·9) h per day in caring activities.

To study wound healing we used a punch biopsy,¹¹ a low-risk technique used extensively in dermatological research. A Miltex Instruments kit was used to create a uniform 3·5 mm full-thickness wound on the non-dominant forearm, roughly 4 cm below the elbow (ventral side). Grove¹² found that individuals show similar time for wound repair across sites.

A hydrocolloid occlusive dressing (DuoDerm; ConvaTec, Princeton, New Jersey, USA) was applied after haemostasis was achieved.¹¹ The dressing was left in place until it was removed by the nurse a week later. The occlusive dressing was reapplied after photographs had been taken and hydrogen peroxide applied, and was left in place until the next photograph; the two groups did not differ in duration of use of the hydrocolloid bandage. Thereafter, the wounds were covered with a standard adhesive dressing, replaced once daily after application of peroxide to cleanse the wound and assess healing. A wound was defined as completely healed when the site no longer foamed after peroxide application.¹¹

Department of Psychiatry (Prof J K Kiecolt-Glaser PhD), **Section of Periodontology, College of Dentistry** (P T Marucha PhD, A M Mercado DMD), and **Departments of Medicine and Medical Microbiology and Immunology** (Prof W B Malarkey MD, Prof R Glaser PhD), **Ohio State University College of Medicine, Columbus, Ohio, USA**

Correspondence to: Prof Janice Kiecolt-Glaser, 1670 Upham Drive, Columbus, Ohio 43210, USA

Beginning a week after biopsy, we photographed the wound every 2-8 days (depending on subject availability) until it was completely healed; the photographer verified that the wounds no longer foamed. Duplicate photographs of the biopsy site were taken each time with a standard-sized dot beside the wound. Slides were digitised by a scanner¹⁴ and imported into NIH Image (National Institutes of Health, Bethesda, MD) on a Macintosh Iici computer. Wound size was expressed as the ratio of the wound area to the dot measurement. The research assistant who measured wounds was unaware of the group to which the subject belonged or time since biopsy. Wound measurements were very reliable for two photographs taken on the same occasion, with an average difference of less than 2%.

8 mL heparinised blood drawn before biopsy was diluted with an equal volume of sterile saline and divided into four polypropylene tubes. One tube had no additions and the others were treated with 100 ng/mL *Salmonella typhimurium* lipopolysaccharide (Sigma, St Louis, MO), 10 ng/mL recombinant TNF- α (BRL, Gaithersburg, MD), or 10 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF; BRL). Whole blood cultures were incubated for 1 h at 37°C in 5% carbon dioxide with gentle rotation. The cultures were centrifuged at 500 g, the erythrocytes were hypotonically lysed, and leucocytes were harvested by centrifugation. RNA was purified, precipitated, then subjected to electrophoresis, blotting, probing for interleukin-1 β , and autoradiography.¹³ On each blot, samples from three subjects were tested along with a standard mRNA aliquot to control for variability in transfer and autoradiography. Data from four subjects (one caregiver, three controls) were lost because of technical problems. A video image of the autoradiographs was examined by densitometry (NIH image 1.55) by an investigator unaware of group or treatment.

The ten-item perceived stress scale,¹⁶ administered at the time of the biopsy and a week later, measured the degree to which subjects perceived their daily life during the preceding week as unpredictable, uncontrollable, and overloading. Subjects rated each item from 0 (never) to 4 (very often).

Analysis of variance (ANOVA) was used to assess group differences. Cytokine data were divided by the standard from each subject's autoradiograph and the resulting value was standardised to the absolute number of monocytes,¹⁷ multiplied by 1000, then subjected to natural log transformation to normalise the distribution.

Results

Complete wound healing took significantly longer in caregivers than in controls (48.7 [2.9] vs 39.3 [3.0] days; $p<0.05$; figure 1). Inclusion of age and income as covariates did not affect the difference between the groups. Wound healing took an average of 9 days (24%) longer in caregivers than in controls.

The differences between the groups in wound size were greatest early in the process of wound repair, and diminished with time (figure 2). Although only one interval's difference was significant (days 9-14, $p<0.05$) because of the small sample, the pattern of group differences was clear.

A multivariate ANOVA that included all three cytokines showed that caregivers produced less interleukin-1 β in response to stimulation than did controls (figure 3). Subsequent univariate ANOVAs showed that caregivers had a significantly poorer response than controls to lipopolysaccharide ($p<0.03$), but not to TNF ($p<0.51$) or GM-CSF ($p<0.34$). Only 22% of control subjects had lipopolysaccharide values below the median, compared with 73% of caregivers ($p<0.05$, χ^2 test).

Caregivers reported significantly more stress on the perceived stress scale than did controls on study entry (20.5 [1.6] vs 13.7 [1.5], $p<0.002$). There was no significant change between the first and second week, or

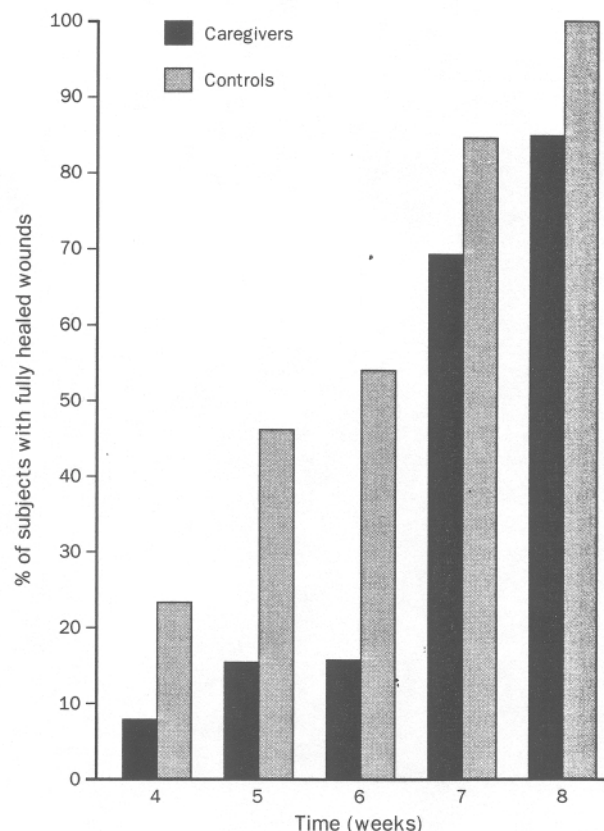


Figure 1: Percentage of caregivers and controls whose wounds had healed with time. Range 24-68 days.

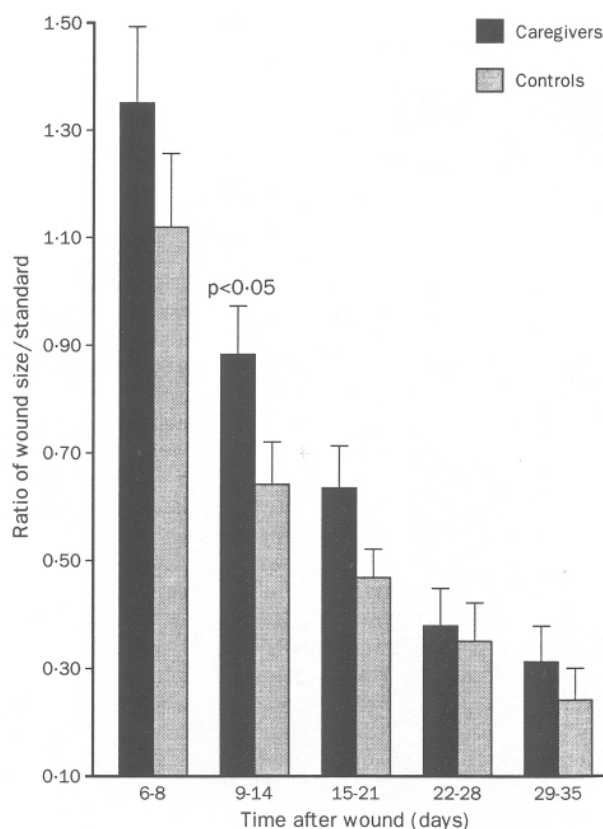


Figure 2: Average wound size during first 5 weeks of study. All wound measurements for a subject during a given week were averaged to provide a single value for the interval for each subject who had not yet healed.

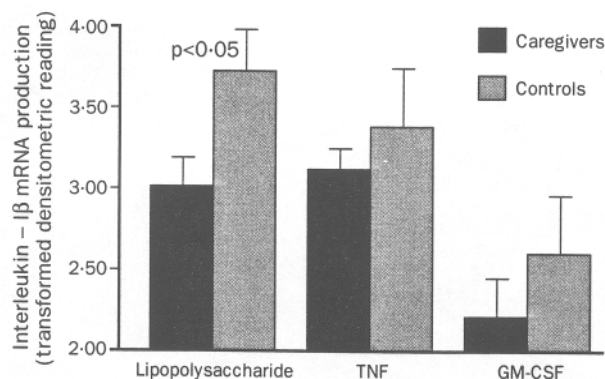


Figure 3: Interleukin-1 β mRNA expression after stimulation with lipopolysaccharide, TNF, and GM-CSF

any interaction between group and time. The mean for the caregivers was more than one standard deviation above the mean for a national sample (ie, within the upper 15% of the distribution for women), whereas controls scored at the mean.¹⁶

Most elderly people take some medication; in our sample, five caregivers and three controls took oestrogen supplements, four and three, respectively, took beta-blockers or calcium-channel blockers, one in each group took diuretics, three caregivers took thyroid supplements, and two anxiolytics. No systematic group differences in medication use were apparent.

Most health-related behaviours did not differ between caregivers and controls, including alcohol use in the preceding week, hours of vigorous physical activity, body mass, weight change within the preceding week, and caffeine intake. All subjects had plasma albumin concentrations and total lymphocyte counts within the normal range, and the groups did not differ for these variables. Smoking is known to impair wound healing; one caregiver and four controls smoked cigarettes. Thus, we found no group differences in health-related behaviours that would have accounted for caregivers' poorer wound healing.

Discussion

Two independent methods for assessment of healing (wound photographs and peroxide response) showed slower repair in caregivers than controls. A finding consistent with the group differences in wound repair was that caregivers also produced less interleukin- β in response to lipopolysaccharide stimulation. Although it is not possible to prove a cause and effect relation between interleukin- β and wound healing in these studies, interleukin- β produced soon after tissue injury can regulate the production, release, and activation of metalloproteinases, which are important in the destruction and remodelling of the connective tissue matrix.^{1,2} Interleukin- β also regulates fibroblast chemotaxis and production of collagen^{1,2} and stimulates production of other cytokines important for wound healing.¹ The greatest differences in wound size were apparent early in the process; the differences in interleukin- β between caregivers and controls suggest

that local production of this cytokine by leucocytes may be one immunological mechanism underlying the observed differences in wound repair.

This study used only a small number of subjects, and the results are preliminary. However, the data are consistent with other studies that have shown immunological differences between stressed dementia caregivers and well-matched controls, particularly the differences in interleukin- β observed in our larger study of 64 caregivers and controls (unpublished).

Previous animal and human studies have shown that stress can increase vulnerability to infectious illness.^{3,4,18} The findings of this study suggest that stress-related alterations in immune function could have additional implications for health beyond infectious disease. Defects in wound repair could have important clinical implications, especially for surgical recovery.

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