

Stress-Related Changes in Proinflammatory Cytokine Production in Wounds

Ronald Glaser, PhD; Janice K. Kiecolt-Glaser, PhD; Phillip T. Marucha, DMD, PhD; Robert C. MacCallum, PhD; Bryon F. Laskowski, BS; William B. Malarkey, MD

Background: Several recent studies have shown that stress markedly delays wound healing. This study assessed the relationship between psychological stress and the secretion of proinflammatory cytokines at an actual wound site, providing in vivo data on the development of local immune responses that are central in the early stages of wound repair.

Methods: To study the dynamics of inflammation, skin blisters were induced on the forearm of 36 women (mean age, 57 years) by suction. After the blister roofs were removed, a plastic template was taped to the arm, and wells were filled with 70% autologous serum in buffer. Specimens were aspirated from blister chamber wells 5 and 24 hours after wounding.

Results: Women with higher perceived stress scores demonstrated significantly lower levels of 2 key cytokines—interleukin 1 α and interleukin 8—at wound sites. In addition, subjects who had low levels of both cytokines after 24 hours reported more stress and negative affect, and they had higher levels of salivary cortisol than those who had high cytokine levels.

Conclusion: Consistent with the evidence that stress delays wound healing, these data suggest a possible mechanism: psychological stress has measurable effects on proinflammatory cytokine production in the local wound environment.

Arch Gen Psychiatry. 1999;56:450-456

From the Departments of Medical Microbiology and Immunology (Drs Glaser, Marucha, and Malarkey and Mr Laskowski), Psychiatry (Dr Kiecolt-Glaser), Periodontology (Dr Marucha), Psychology (Dr MacCallum), and Internal Medicine (Dr Malarkey), The Institute for Behavioral Medicine Research (Drs Glaser, Kiecolt-Glaser, Marucha, MacCallum, and Malarkey), and the Comprehensive Cancer Center (Drs Glaser, Kiecolt-Glaser, Marucha, and Malarkey), The Ohio State University, Columbus.

MANY STUDIES^{1,2} in psychosomatic medicine have shown that greater fear or distress before surgical procedures is associated with poorer outcomes, including longer hospital stays, more postoperative complications, and higher rates of readmission to a hospital. One key psychological mechanism is suggested by evidence that stress slows wound healing. For example, in women caring for a relative with Alzheimer disease, a small, standardized dermal wound took 24% longer to heal than in well-matched controls.³ Further research demonstrated that even a transient, commonplace stressor—academic examinations—could substantially delay mucosal wound repair: wounds placed on the hard palate 3 days before a major test healed an average of 40% slower than those made in the same persons during summer vacation.⁴

Wound repair progresses through overlapping stages.⁵ In the initial inflammatory phase, vasoconstriction and blood coagulation are followed by platelet activation and the release of platelet-derived growth factors.⁶ The factors act as che-

moattractants for the migration of phagocytes (neutrophils and monocytes) to the site, starting the proliferative phase that involves the recruitment and replication of cells necessary for tissue regeneration and capillary regrowth. Wound remodeling—the final step—may continue for weeks or months. The healing process is a cascade, and success in later stages is highly dependent on initial events.^{5,7}

Immune function plays a key role early in this cascade. Proinflammatory cytokines such as interleukin (IL) 1 and tumor necrosis factor (TNF) are essential to this effort; they help protect against infection and prepare injured tissue for repair by enhancing phagocytic cell recruitment and activation.⁷ Furthermore, cytokines released by recruited cells regulate the ability of fibroblasts and epithelial cells to remodel the damaged tissue. Interleukin 1 produced early after tissue injury can regulate the production, release, and activation of metalloproteinases that are important in the destruction and remodeling of the wound; IL-1 also regulates fibroblast chemotaxis and the production of collagen. Furthermore, IL-1 stimulates the production of other cyto-

SUBJECTS AND METHODS

SUBJECTS

The subjects, 36 postmenopausal women (mean \pm SD age, 57.2 \pm 6.6 years) responded to announcements offering \$200 for a study on wound healing; notices had been placed in community, hospital, and university newspapers, as well as senior citizen centers. The participants were not selected on the basis of any stress criteria and were not screened for psychiatric illness. Based on the substantial relationships observed between stress and wound healing, even within the control group in a previous dermal wound study,³ these women were recruited through the same sources (and with the same health criteria) to provide parallel data on mechanisms. Women who met criteria following a 15- to 20-minute telephone interview were sent a detailed letter describing the protocol. The average education was partial college; 16 were married, 13 were separated or divorced, and the rest were widowed ($n = 5$) or single ($n = 2$). Subjects were excluded if they reported a history of diabetes mellitus or peripheral vascular disease, difficulties with wound healing, the use of anti-inflammatory medication or other medications with obvious immunologic consequences, or immunologically related health problems, eg, cancer, autoimmune disease, or recent surgical treatment. In addition, smokers were excluded, as were women who reported drinking 10 or more alcoholic beverages per week. The 17 women taking estrogen supplements, medroxyprogesterone acetate, or both, were evaluated during the estrogen-only phase of their cycle. Data from 4 women were not included in the final analyses; 2 had no blister chamber data after 24 hours due to leakage, 1 subject's complete blood cell count was aberrant, and 1 woman's baseline cortisol level fell more than 4 SDs above the mean. The Ohio State University Biomedical Research Review Committee approved the project; all subjects gave written informed consent before the initial blood specimen was drawn (**Table 1**).

SUCTION BLISTER PROTOCOL

Our suction blister protocol (Table 1) was taken directly from the one used in several studies conducted at the

National Institute of Allergy and Infectious Diseases¹³⁻¹⁵ and used the same commercial suction blister device (Neuro-Probe, Cabin John, Md), described in detail elsewhere.¹⁵ To assess the early phase of the inflammatory response to wounding *in vivo*, a plastic template was taped to the volar surface of the nondominant forearm; a vacuum of 350 mm Hg was applied through a pump attached to a regulator until blisters formed (1-1½ hours). This gentle suction separated the dermoepidermal junction, creating 8 sterile 8-mm blisters, each roughly the size of a pea. After the fluid was drained from each blister (0.05-0.1 mL) with a syringe, the blister roof (the epidermis) was removed with sterile scissors, and a plastic template was placed over the blister wounds. Using a syringe, the template wells were filled with 0.8 to 1.0 mL of 70% autologous serum in a Hanks' balanced salt solution,¹⁵ and the top was sealed with sterile tape. Five hours after the blisters were raised, the autologous serum-buffer solution was aspirated from half the wells with a syringe; the remaining solution was aspirated from the other 4 wells after 24 hours. Cells in the fluid were counted and then separated by centrifugation.

PSYCHOLOGICAL DATA AND HEALTH-RELATED BEHAVIORS

The 10-item Perceived Stress Scale (PSS)¹⁶ measured the degree to which subjects perceived their daily life during the previous week as unpredictable, uncontrollable, and overloading on items rated from 0 (never) to 4 (very often). The Cronbach α was .91 in this sample. Although overlapping with measures of psychological distress (particularly depressive symptoms), PSS scores are better predictors of physical symptoms and health services use.¹⁶

The Positive and Negative Affect Schedule, a 20-item scale, measures 2 psychometrically independent dimensions of emotional experience that have different correlates.¹⁷ Negative affect is strongly related to symptoms such as depression, as well as perceived stress and health complaints. Positive affect is associated with social activity, exercise, and health complaints.¹⁷ The Cronbach α was .90 and .89 for negative and positive affect, respectively.

Continued on next page

kines important for wound healing, including IL-2, IL-6, and IL-8. Accordingly, IL-1 deficits early in the wound repair cascade can have adverse consequences later in the process.

The dysregulation of glucocorticoid secretion provides 1 obvious mechanism through which stress can disrupt the cascade. For example, mice treated with glucocorticoids showed impairments in the induction of IL-1 and TNF, as well as defects in wound repair.⁵ Mice subjected to restraint stress had serum corticosterone levels more than 4 times those of unstressed mice, and a dermal wound healed 27% slower in the former mice than in the latter.⁸ When the glucocorticoid receptors of restraint-stressed animals were blocked with RU 40555, their healing rates were comparable to those of control mice.

In contrast to the generally adverse effects of glucocorticoids, growth hormone (GH) can enhance wound healing.⁹ This hormone serves as a macrophage activa-

tor; among its activities, it stimulates monocyte migration and amplifies mechanisms for protection from infection.^{10,11}

Early events in wound healing, particularly in the first 24 hours, represent a critical period.^{7,12} Skin blister wounds allow an investigator to monitor the kinetics of this key phase *in vivo*.¹³ We hypothesized that higher levels of psychological stress would be associated with impairments in the local inflammatory response.

RESULTS

DISCOMFORT DURING THE SUCTION BLISTER PROTOCOL

The blister procedures produced minimal pain or discomfort. Using a 0 to 6 scale, only 4 women rated the highest level of discomfort experienced during the hour

The Psychiatric Epidemiological Research Inventory Life Events Scale¹⁸ provided data on the number and types of major life changes in the past year. Subjects were asked whether each event was “negative” (detrimental) or “positive” (beneficial).

Health-related behaviors, assessed at entry to the General Clinical Research Center, included recent medication use, hours of sleep in the past 24 hours and the past 3 days, and recent alcohol intake.¹⁹ Two questions assessed exercise.²⁰ The 36-item RAND Health Survey²¹ provided a non-disease-specific measure of functioning and well-being with excellent normative data.

IMMUNOLOGIC AND ENDOCRINOLOGIC STUDIES

Blood specimens were collected in EDTA tubes (for plasma) and serum tubes (Vacutainer; Becton Dickinson Labware, Franklin Lakes, NJ). After the latter were allowed to clot at room temperature for 30 minutes, they were centrifuged for 10 minutes at 2700 rpm; serum was removed and stored at -86°C until assayed. Blister fluid was centrifuged for 30 seconds to extract any cells in a pellet, and the supernatant was removed and stored at -86°C until assayed.

Specimens and standards were set up in duplicate on human IL-1 α and IL-8 kits (Quantikine; R&D Systems, Minneapolis, Minn). Specimens were incubated for 1 hour on the plates, and enzyme-linked immunosorbent assays were performed according to the manufacturer's instructions. All chamber fluid specimens used for assaying IL-1 α had to be diluted 1:2 to fall within the standards range; data for IL-1 α in serum specimens were not available because of the sensitivity of the kits. For the IL-8 enzyme-linked immunosorbent assay, 5- and 24-hour chamber fluid specimens were diluted 1:10 and 1:75, respectively, and serum specimens were used undiluted.

Plasma cortisol, GH, and salivary cortisol were assayed using chemiluminescent techniques (Nichols Institute, San Juan Capistrano, Calif). The saliva obtained from a dental cotton roll was lyophilized and reconstituted at a 5-fold higher concentration for the afternoon and evening values to fall within detection limits. These assays have excellent sensitivity and specificity with intra-assay and interassay coefficients of variation of less than 8%.

STATISTICAL METHODS

Several primary research questions involved the relationships between continuous independent variables and repeated measures of dependent variables. These relationships were analyzed using repeated-measures regression models,²² which are generalizations of the standard multiple regression model to incorporate repeated measures of dependent variables.^{23,24} For the analysis of immune measures, dependent variables were repeated measures (5- and 24-hour specimens) of IL-1 α and IL-8 levels and cell numbers from peripheral blood and blister wells. Independent variables were PSS scores and levels of 2 key hormones—cortisol and GH—measured from plasma and blister fluid specimens. The same regression method was used to study blood pressure and heart rate measured on 4 occasions during the suction blister procedure, with perceived stress as the sole predictor. Analogous to the analysis of variance, this regression method partitions the variance of a dependent variable into within-subjects effects, consisting of the effects of time and interactions of time with the continuous independent variables, and between-subjects effects, consisting of the main effects of the continuous independent variables. A significant effect of time implies a significant change in the mean level of the dependent variable with time. A significant interaction of a continuous independent variable with time implies that the pattern of change with time varies with the level of the independent variable. A significant main effect of a continuous independent variable implies that the predictor explains a statistically significant variation in differences among subjects collapsed across time.

Another set of analyses was conducted to study characteristics of subjects producing high vs low levels of cytokines at wound sites to identify more homogeneous or distinct groups. Median splits were applied to both IL-1 α and IL-8 levels, and subjects falling into the high or low category on both cytokines were designated as high or low producers, respectively. Analyses of variance were used to analyze differences between these 2 subgroups on psychological and endocrine variables, as well as measures of health behavior. All tests were 2-tailed, using a .05 α level.

when the blisters were raised and de-roofed as high as 3 (“distressing”), 12 (33%) said it was discomforting (score of 2), 17 (47%) rated it mild (score of 1), and 3 (8%) gave ratings of 0 (none). Thereafter, with the blister chamber in place, the site was rated as 0 by 31 (86%), and the remainder rated it as 1. Similarly, cardiovascular data collected by nurses (**Table 2**) did not suggest the procedure was stressful.

REGRESSION ANALYSES: BLISTER CHAMBERS AND PERIPHERAL BLOOD

The blister chamber fluid analyses used the data from 2 chambers as replicates. The correlations between pairs of chambers demonstrated good reproducibility, with $r = 0.97$ and $r = 0.92$ for IL-1 α at 5 and 24 hours, respectively; $r = 0.90$ and $r = 0.72$ for IL-8 for the same periods; and $r = 0.84$ and $r = 0.76$ for the number of cells that had

migrated into the chambers from the wound site ($P < .001$ for all). The mean of each pair was used for subsequent analyses.

The 2 baseline measures for cortisol and GH levels, 1 from plasma and 1 from the blister (interstitial) fluid, were significantly correlated (for GH, $r = 0.67$, and for cortisol, $r = 0.67$; $P < .001$ for both). For GH, the blister fluid mean \pm SD level was 0.4 ± 0.4 ng/mL, compared with 2.5 ± 3.2 ng/mL in plasma, whereas the respective values for cortisol were 2.6 ± 1.0 $\mu\text{g/dL}$ and 15.3 ± 4.1 $\mu\text{g/dL}$. To reduce the number of variables in the regression analyses, we combined the 2 baseline values for each hormone by converting them to z scores and averaging them.

The number of cells in blister chamber fluid specimens increased markedly ($F_{1,30} = 41.35$; $P < .001$), with a mean \pm SD (\log_{10}) of 3.5 ± 1.1 cells per milliliter and 5.8 ± 0.5 cells per milliliter at 5 and 24 hours, respectively. The number of cells that accumulated in blister

Table 1. Sampling Schedule*

Study Day	Time	Procedure
1-3 Before	6:00 PM	Peripheral blood specimen drawn for autologous serum–buffer solution
1	7:00 AM	Admission to General Clinical Research Center (GCRC); baseline blood pressure; salivary cortisol specimen; questionnaires administered; and peripheral blood specimen for cortisol, GH, IL-1 α , and IL-8 levels
	7:30 AM	Begin raising suction blisters
	7:45 AM	Blood pressure measurement
	8:00 AM	Blood pressure measurement
	8:30 AM	Blister (interstitial) fluid aspirated for cortisol and GH levels; blisters de-roofed; blister chamber taped to arm, and wells filled with autologous serum–buffer solution; and blood pressure measurement
1	1:30 PM	Contents of 4 blister wells aspirated for cells and IL-1 α and IL-8 levels; peripheral blood specimen drawn for cortisol, GH, and cytokine levels; salivary cortisol specimen obtained; and subject sent home with blister chamber taped to arm
	2	8:30 AM

*GH indicates growth hormone; IL, interleukin.

wells was not associated with stress or GH or cortisol levels ($F < 1.00$; $P > .44$ for all). When the CD13 monoclonal antibody was used to identify myeloid cells by flow cytometry in the 24-hour blister chamber specimens of 17 subjects, 0.92 of the cells were neutrophils, 0.023 were macrophages (MO2 monoclonal antibody, $n = 13$), and 0.032 were T lymphocytes (CD3 monoclonal antibody, $n = 12$).

Stress was significantly associated with the production of both cytokines at the wound site (**Figure 1** and **Figure 2**). Tests of between-subjects effects showed that baseline cortisol levels had no significant relationships to either cytokine ($P > .21$ for all); substituting baseline salivary cortisol values in the analyses did not alter the nonsignificant contribution of this hormone in this set of analyses (but see below, relationships with cortisol data spanning 24 hours). Growth hormone levels, however, were a significant predictor of IL-8 levels in the blister chamber fluid ($F_{1,32} = 4.81$; $P < .04$); in addition, a significant interaction for GH and time showed that higher levels of GH were associated with higher levels of IL-8 after 5 hours, whereas higher levels of GH were associated with lower levels of IL-8 after 24 hours ($F_{1,32} = 5.00$; $P = .03$). The GH level was not related to the IL-1 α level ($F < 1.00$; $P > .36$ for all). Finally, levels of IL-1 α and IL-8 in blister chambers were significantly related after 24 hours ($r = 0.58$; $P < .001$), and the level of IL-8 in blister chamber fluid specimens after 24 hours was correlated with the accumulation of cells in chambers ($r = 0.73$; $P < .001$).

In contrast to the increased amount of IL-8 in the blister chamber fluid, serum IL-8 levels from peripheral

Table 2. Changes in Heart Rate and Blood Pressure During the Suction Blister Protocol (N = 36)*

Variable	Heart Rate, Beats/min	Blood Pressure, mm Hg	
		Systolic	Diastolic
Baseline, GCRC entry	76.8 \pm 9.9	126.6 \pm 13.8	72.1 \pm 11.6
After blisters started, min			
15	70.8 \pm 8.2	121.7 \pm 12.9	71.1 \pm 9.5
30	68.5 \pm 8.4	121.2 \pm 10.6	72.6 \pm 10.5
After chamber was in place	68.1 \pm 7.5	123.4 \pm 14.7	72.6 \pm 9.8
$F_{3,32}$	7.27	2.95	0.61
P	.001	.05	.62

*Values are given as mean \pm SD. Perceived Stress Scale scores were not significantly associated with any of the 3 cardiovascular measures, either in main effects or interactions with time ($P > .12$ for all). GCRC indicates General Clinical Research Center.

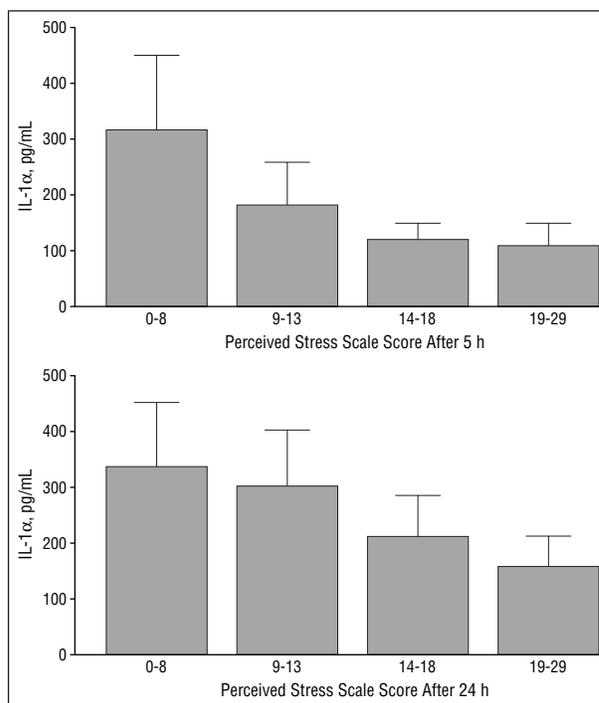


Figure 1. Perceived Stress Scale scores were divided into quartiles to illustrate the relationship between stress and interleukin 1 α (IL-1 α) production after 5 (top) and 24 (bottom) hours ($n = 36$). The increase in IL-1 α levels between 5 and 24 hours did not reach significance ($F_{1,32} = 3.60$; $P = .07$). Higher stress was associated with lower production of IL-1 α ($F_{1,32} = 5.73$; $P < .03$).

blood showed no change with time ($F < 1.00$; $P = .53$) and no significant association with stress or either hormone. Similarly, we found no relationship between leukocyte counts in peripheral blood (from the complete blood cell count) and the number of cells in the blister chambers after 5 ($r = 0.06$) or 24 hours ($r = -0.02$).

PSYCHOLOGICAL AND HEALTH BEHAVIOR DATA

Most middle-aged and older adults take some medication; in our sample, 17 women took estrogen supple-

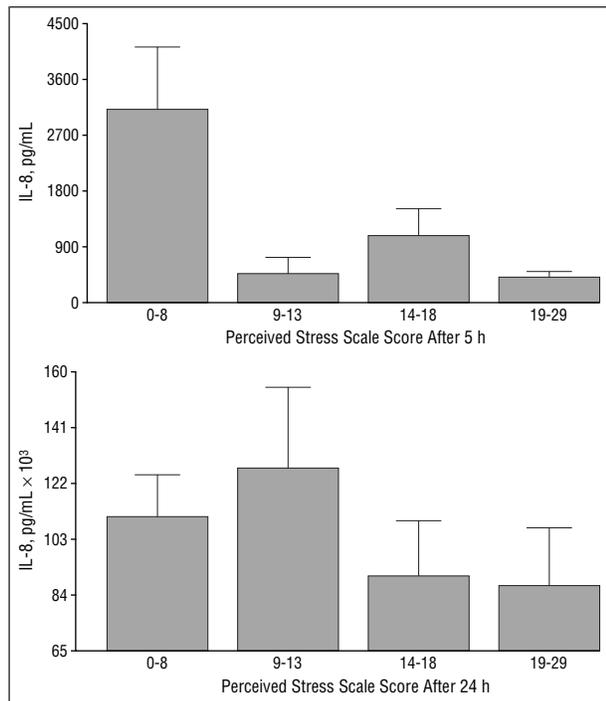


Figure 2. Perceived Stress Scale scores were divided into quartiles to illustrate the relationship between stress and interleukin 8 (IL-8) production at 5 (top) and 24 (bottom) hours ($n = 36$). Higher stress was generally associated with lower production of IL-8 ($F_{1,32} = 5.31$; $P < .03$); the interaction between stress and time reflected some variation in the effect of stress between the 2 intervals ($F_{1,32} = 4.56$; $P < .04$).

ments, 5 took thyroid supplements, and 2 used prescription diuretic medications. We found no systematic differences related to any type of medication. In particular, comparisons between the 17 women taking estrogen and the remainder of the sample showed no difference on PSS scores ($F < 1.00$; $P = .44$). When estrogen replacement therapy was included as a between-subjects variable in the regression analyses for the prediction of immune assays, its effect was not significant, and it did not alter the findings for stress.

Health-related behaviors were not correlated with cytokine levels. All subjects were within the reference ranges for plasma albumin levels and total lymphocyte counts. Thus, we found no significant relationships between health habits and immune data.

CHARACTERISTICS OF SUBJECTS PRODUCING HIGH OR LOW LEVELS OF CYTOKINES AT WOUND SITES

Subjects who had low cytokine levels reported more stress, more negative affect, and a marginally higher number of adverse life events compared with those with high cytokine levels (**Table 3**), with no differences in positive affect. Consistent with their greater distress, women with low cytokine levels had higher levels of salivary cortisol than women with high cytokine levels (**Figure 3**, top). The group-by-time interaction in plasma cortisol levels reflected some variation in the relationship across the 3 time points (Figure 3, bottom).

Women with low and high cytokine levels did not differ on GH levels ($F_{1,25} = 1.55$; $P = .22$). The group-by-

Table 3. Psychological Test Scores From Subjects With High and Low Cytokine Levels*

Test	Cytokine Levels, Mean \pm SD		$F_{1,27}$	P
	Low (n = 13)	High (n = 15)		
Perceived Stress Scale	18.1 \pm 7.8	11.9 \pm 6.4	5.37	.03
Negative affect ratings, PANAS	21.0 \pm 8.5	15.1 \pm 4.7	5.26	.03
Positive affect ratings, PANAS	33.1 \pm 9.0	35.9 \pm 6.6	0.09	.36
Negative life events, PERI	2.4 \pm 2.3	1.1 \pm 1.3	3.66	.07

*PANAS indicates Positive and Negative Affect Schedule; PERI, Psychiatric Epidemiological Research Inventory.

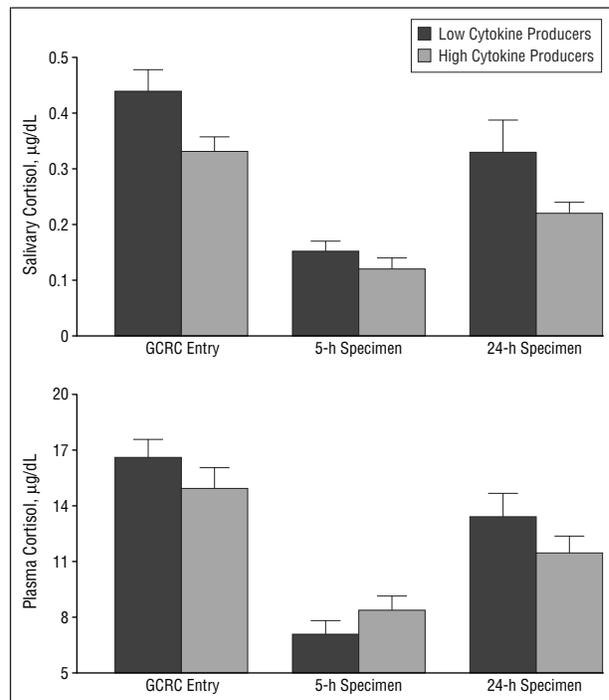


Figure 3. Salivary cortisol (top) and plasma cortisol (bottom) measurements from subjects who had high or low levels of both interleukin (IL) 1 α and IL-8 after 24 hours. Subjects with low cytokine levels ($n = 12$) had higher levels of salivary cortisol than those with high levels ($n = 12$) ($F_{1,22} = 4.29$; $P < .05$); there was significant diurnal variation ($F_{2,44} = 27.54$; $P < .001$), without a significant interaction between group and time ($F_{2,44} = 1.26$; $P = .29$). Although plasma cortisol levels showed a nonsignificant group effect ($F < 1.00$; $P = .59$), there was a significant group-by-time interaction ($F_{2,50} = 3.44$; $P < .05$) and significant diurnal variation ($F_{2,50} = 68.90$; $P < .001$). GCRC indicates General Clinical Research Center.

time interaction was similarly not significant ($F_{2,24} = 2.06$; $P = .15$).

Health habits and health perceptions were not reliably related to cytokine production. Among the health behaviors described earlier, none differed between groups, including estrogen status ($\chi^2_1 = 1.29$; $P = .45$). The 2 groups did not differ on any of the physical health dimensions of the RAND Health Survey²¹ ($P > .88$ for both). Although women with high cytokine levels were older than those with low cytokine levels by a mean \pm SD of 5.4 \pm 3.8 years (60.2 \pm 8.1 years vs 54.8 \pm 4.3 years, respectively, $F_{1,27} = 4.53$; $P < .05$), controlling for age did not alter group differences.

Psychological stress can have measurable adverse consequences for key immunologic events at wound sites. Women who reported more stress produced significantly lower levels of 2 proinflammatory cytokines important for the early stages of wound healing—IL-1 α and IL-8. As expected, immunologic changes in peripheral blood did not reflect these events at the wound site. In accord with recent studies^{3,4,8} that have revealed large and significant relationships between stress and wound healing, these data suggest 1 obvious mechanism: stress can delay the appearance of proinflammatory cytokines early in the wound repair cascade.

Stress-induced elevations in glucocorticoid levels can alter the carefully regulated dynamic system that controls development of the inflammatory response, suppressing IL-1 α and TNF production.^{5,25} Consistent with these data, we found that women who produced low cytokine levels reported more stress and more negative affect than women who produced high cytokine levels, and the former also had higher levels of salivary cortisol. Findings for plasma cortisol were similar but showed less consistent relationships with time; the 2 may differ because plasma cortisol measurements provide data on protein-bound and -unbound fractions of the hormone, whereas salivary cortisol levels represent only the unbound, “free” fraction.²⁶

One limitation of the study is the relative frequency of endocrine specimens. Elevations in cortisol levels in response to novel or threatening stimuli have been reported repeatedly.^{26,27} Thus, subjects’ initial responses to the protocol may have overshadowed more persistent stress-related differences, reflected in the heightened values observed at baseline compared with those obtained after 24 hours.

Similarly, 1 explanation for the unexpected interaction between time and GH levels in IL-8 analyses may be related to the changes in the kinetics of GH during the period in question; most GH secretion occurs at night during deep sleep,⁹ a period not assessed in this study. The positive relationship between baseline GH and IL-8 after 5 hours is likely to be more reliable than the negative relationship between baseline GH and IL-8 levels after 24 hours because of the shorter interval between the former measures and because the highest secretion of GH occurs between the 5- and 24-hour measurements of IL-8.

The present observations on the kinetics of the local inflammatory response are in accord with previous studies using suction blister wounds. For example, in previous work,¹³ the migration of leukocytes (primarily neutrophils) into the chamber fluid was detectable within 5 hours and appeared to plateau at 16 to 24 hours. Similarly, other studies found that IL-1 α levels did not increase significantly, in contrast to those of IL-8, a potent neutrophil chemoattractant, which showed significant increases in blister chamber fluid during the first 24 hours.¹⁴ Several cell types appear to produce IL-8, including peripheral blood neutrophils, and the appearance of neutrophils in the chambers was correlated with IL-8 secretion.¹⁴

A disruption of immunologic events in the local wound environment can have clinically important con-

sequences. In 3 studies^{3,4,8} that assessed the repair of standardized wounds, the stress-related delays in healing ranged from 24% to 40%, and the effect sizes (using a squared simple correlation as a measure of the proportion of variance in healing time accounted for by stress) were between $r^2 = 0.30$ and $r^2 = 0.74$. Unquestionably, these effects are not just statistically significant but large in a substantive sense.

The stress-related deficits in local cytokine production and wound repair are consistent with the broad psychosomatic medicine literature addressing psychological influences on postsurgical recuperation.²⁸ Among the more than 200 studies^{1,29,30} published in the past 3 decades on presurgical intervention, beneficial effects have included decreased anxiety and stress, reductions in hospital stay, fewer postoperative complications, better treatment compliance, less pain and use of analgesic medications, and alterations in various physiological indices. Given the substantial consequences of stress for wound repair, even a small diminution in anxiety could have substantial clinical consequences, both directly and indirectly.²⁸

In this study, the women’s mean \pm SD PSS score was 14.4 ± 7.3 , virtually identical to our control group’s mean in our first wound study³; furthermore, the value falls at the mean for women in data from a national sample.¹⁶ Accordingly, these data demonstrate that stress, even within a normal range, can be associated with alterations in the local production of proinflammatory cytokines. Much larger effects are likely among clinically depressed persons, based on the well-documented links between depression and immune dysregulation, as well as depression and cortisol levels.^{31,32}

Indeed, recent data from a well-controlled study³³ showed that depressive symptoms were significant predictors of infection-related readmission to a hospital following coronary artery bypass graft surgery, illustrating the importance of identifying and treating at-risk patients before surgery. Psychological or pharmacological presurgical interventions could have a notable effect on the quality of life for patients and families, as well as substantial economic savings,³³ areas of obvious importance for future work.

Accepted for publication February 9, 1999.

This work was supported in part by research grants K02 MH01467 and R37 MH42096 from the National Institutes of Health and MO1-RR-0034 from the General Clinical Research Center, Bethesda, Md; and Core Grant CA16058 from The Ohio State University Comprehensive Cancer Center, Columbus.

Reprints: Ronald Glaser, PhD, Department of Medical Microbiology and Immunology, The Ohio State University, 2175 Graves, 333 W 10th Ave, Columbus, OH 43210 (e-mail: Glaser.1@osu.edu).

REFERENCES

1. Contrada RJ, Leventhal EA, Anderson JR. Psychological preparation for surgery: marshalling individual and social resources to optimize self-regulation. In: Maes S, Leventhal H, Johnston M, eds. *International Review of Health Psychology*. Chichester, England: John Wiley & Sons Inc; 1994:219-266.

2. Johnston M. Impending surgery. In: Fisher S, Reason J, eds. *Handbook of Life Stress, Cognition and Health*. New York, NY: John Wiley & Sons Inc; 1988:79-100.
3. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. *Lancet*. 1995;346:1194-1196.
4. Marucha PT, Kiecolt-Glaser JK, Favagehi M. Mucosal wound healing is impaired by examination stress. *Psychosom Med*. 1998;60:362-365.
5. Hübner G, Brauchle M, Smola H, Madlener M, Fassler R, Werner S. Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine*. 1996;8:548-556.
6. Van De Kerkhof PCM, Van Bergen B, Spruijt K, Kuiper JP. Age-related changes in wound healing. *Clin Exp Dermatol*. 1994;19:369-374.
7. Lowry SF. Cytokine mediators of immunity and inflammation. *Arch Surg*. 1993; 28:1235-1241.
8. Padgett DA, Marucha PT, Sheridan JF. Restraint stress slows cutaneous wound healing in mice. *Brain Behav Immun*. 1998;12:64-73.
9. Veldhuis JD, Iranmanesh A. Physiological regulation of the human growth hormone (GH)-insulin-like growth factor type I (IGF-I) axis: predominant impact of age, obesity, gonadal function, and sleep. *Sleep*. 1996;19(10 suppl):S221-S224.
10. Sabharwal P, Zwilling B, Glaser R, Malarkey WB. Cellular immunity in patients with acromegaly and prolactinomas. *Prog Neuroendocrin Immunol*. 1992;5:120-125.
11. Zwilling BS. Neuroimmunomodulation of macrophage function. In: Glaser R, Kiecolt-Glaser JK, eds. *Handbook of Human Stress and Immunity*. San Diego, Calif: Academic Press Inc; 1994:53-76.
12. Barbul A. Immune aspects of wound repair. *Clin Plast Surg*. 1990;17:433-442.
13. Kuhns DB, DeCarlo E, Hawk DM, Gallin JI. Dynamics of the cellular and humoral components of the inflammatory response elicited in skin blisters in humans. *J Clin Invest*. 1992;89:1734-1740.
14. Kuhns DB, Gallin JI. Increased cell-associated IL-8 in human exudative and A23187-treated peripheral blood neutrophils. *J Immunol*. 1995;154:6556-6562.
15. Zimmerli W, Gallin JI. Monocytes accumulate on Rebuck skin window cover-slips but not in skin chamber fluid: a comparative evaluation of two in vivo migration models. *J Immunol Methods*. 1987;96:11-17.
16. Cohen S, Williamson GM. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, eds. *The Social Psychology of Health*. Beverly Hills, Calif: Sage Publications; 1988:31-67.
17. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol*. 1988;54: 1063-1070.
18. Dohrenwend BS, Krasnoff L, Askenasy AR, Dohrenwend BP. Exemplification of a method for scaling life events: the PERI Life Events Scale. *J Health Soc Behav*. 1978;19:205-229.
19. Kiecolt-Glaser JK, Glaser R. Methodological issues in behavioral immunology research with humans. *Brain Behav Immun*. 1988;2:67-78.
20. Washburn RA, Adams LL, Hale GT. Physical activity assessment for epidemiologic research: the utility of two simplified approaches. *Prev Med*. 1987;16:626-646.
21. Ware JE, Sherbourne CD. The MOS 36-Item-Short-Form Health Survey (SF-36), I: conceptual framework and item selection. *Med Care*. 1992;30:473-483.
22. *SPSS 8.0 for Windows*. Chicago, Ill: SPSS; 1997.
23. Cohen J, Cohen P. *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates Inc Publishers; 1983.
24. Keppel G, Zedeck S. *Data Analysis for Research Designs*. New York, NY: WH Freeman & Co; 1989.
25. DeRijk R, Michelson D, Karp B, Petrides J, Galliven E, Deuster P, Paciotti G, Gold PW, Sternberg EM. Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 β (IL-1 β), IL-6, and tumor necrosis factor- α (TNF- α) production in humans: high sensitivity of TNF- α and resistance of IL-6. *J Clin Endocrinol Metab*. 1997;82:2182-2192.
26. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology*. 1994; 19:313-333.
27. Seeman TE, Robbins RJ. Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocr Rev*. 1994;15:233-259.
28. Kiecolt-Glaser JK, Page GG, Marucha PT, MacCallum RC, Glaser R. Psychological influences on surgical recovery: perspectives from psychoneuroimmunology. *Am Psychol*. 1998;53:1209-1218.
29. Johnston M, Voge C. Benefits of psychological preparation for surgery: a meta-analysis. *Ann Behav Med*. 1993;15:245-256.
30. Johnston M, Wallace L. *Stress and Medical Procedures*. Oxford, England: Oxford University Press; 1990.
31. Miller AH. Neuroendocrine and immune system interactions in stress and depression. *Psychiatr Clin North Am*. 1998;21:443-463.
32. Keller SE, Shiflett SC, Schleifer SJ, Bartlett JA. Stress, immunity, and health. In: Glaser R, Kiecolt-Glaser JK, eds. *Handbook of Human Stress and Immunity*. San Diego, Calif: Academic Press Inc; 1994:217-244.
33. Scheier MF, Matthews KA, Owens JF, Schulz R, Bridges MW, Magovern GJ, Carver CS. Optimism and rehospitalization following coronary artery bypass graft surgery. *Arch Intern Med*. 1999;159:829-835.